DEVELOPMENT &
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CRDEC-TR-299

# STRUCTURE DETERMINATION OF COMPOUND 34



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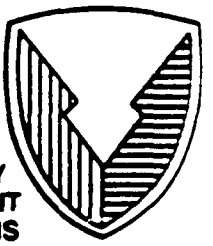
RESEARCH DIRECTORATE

September 1991







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Compound 34 is an indicator used in the M19 Chemical, Biological, and Radiological sampling and analyzing kit for detecting G-agents. For the past 25 yr, numerous discrepancies have existed in both the chemical name and the chemical structure of this compound. Using one- and two-dimensional NMR techniques together with mass spectrometry (MS) and derivatization and degradation of the actual material, the structure of Compound 34 was determined unambiguously. In addition, thin layer chromatography (TLC) and $^{1}H$ NMR were used to determine the purity of the U.S. Army Chemical Research, Development and Engineering Center sample.

**14. SUBJECT TERMS**

| | | |
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PREFACE

The work described in this report was authorized under Project No. 1L162622A553, CB Defense/General Investigation (Operation Desert Shield). This work was started in October 1990 and completed in December 1990.

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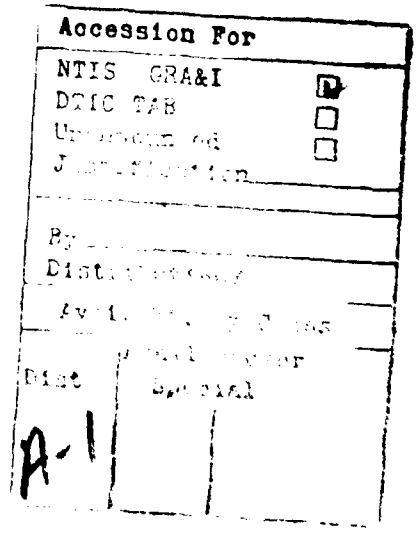
This report has been approved for release to the public.



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# STRUCTURE DETERMINATION OF COMPOUND 34

## 1. INTRODUCTION

In support of a Detection Directorate, U.S. Army Chemical Research, Development and Engineering Center (CRDEC), effort to deliver chemical agent detector kits to Saudi Arabia, the Research Directorate, CRDEC, was requested to determine if a sample of Compound 34, an indicator used in the M19 Detector Kit, met the criteria of its Military Specification (MIL-T-51023D, 29 December 1967). During the course of these investigations, questions arose regarding the purity of the CRDEC sample lots and the accepted structure of Compound 34.

Over the past several years, many new one- and two-dimensional NMR experiments have been developed that allow the NMR spectroscopist to make unequivocal assignments of chemical structure. COrrelated SpectroscopY (COSY), one and two-dimensional Nuclear Overhauser Enhancement SpectroscopY (NOESY), and HETeronuclear CORrelated (HETCOR) spectroscopy are just a few of the techniques in the NMR spectroscopist's arsenal of experiments that can be used to help assign the structure of a compound. Using these techniques with mass spectrometry (MS) to study Compound 34 and two of its derivatization/degradation products, we were able to assign the structure of Compound 34 unambiguously.

## 2. BACKGROUND

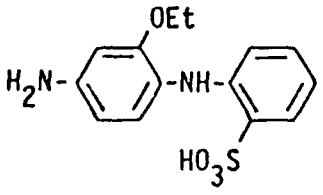
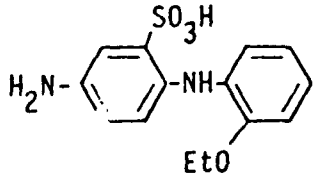
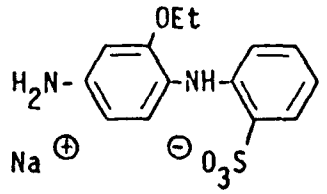
Compound 34 was studied in the early 1950's and found to be an excellent colorimetric indicator for G-agents when used in conjunction with sodium pyrophosphate peroxide in the Schoenemann reaction.[1,2] Currently, Compound 34 is a reagent in the M19 Chemical, Biological, and Radiological (CBR) Agent Sampling and Analyzing Kit.[3] This kit is used to detect and identify CW agents, to perform preliminary processing of suspect CBR samples, and to delineate contaminated areas. The M19 was fielded in the Persian Gulf to be used under Project Desert Shield.

A review of the literature of the past 30 yr revealed discrepancies in the nomenclature for Compound 34; eight similar but different names were found (Table 1). Most of the reports did not give structures for Compound 34; however, three different structures were found to have been reported (Table 2). The name given for Compound 34 in the Operator's Manual for the M19 Detector Kit (TM 3-6665-205-10/2) indicates that the primary amino and ethoxy moieties are on the same ring, with the sulfonic acid group on the ring by itself. On the other hand, the structure given in the same technical manual shows the ethoxy on the ring by itself and the sulfonic acid moiety on the ring with the primary amino group. One report and several notebook entries even showed Compound 34 as a sodium salt. In addition, hearsay indicated that the lots on hand at CRDEC were "around 30% pure" and that this was the reason Compound 34 had to be recrystallized before being made into tablets for use in the detector kits.[4]

Table 1. Nomenclature for Compound 34 Found in the Literature

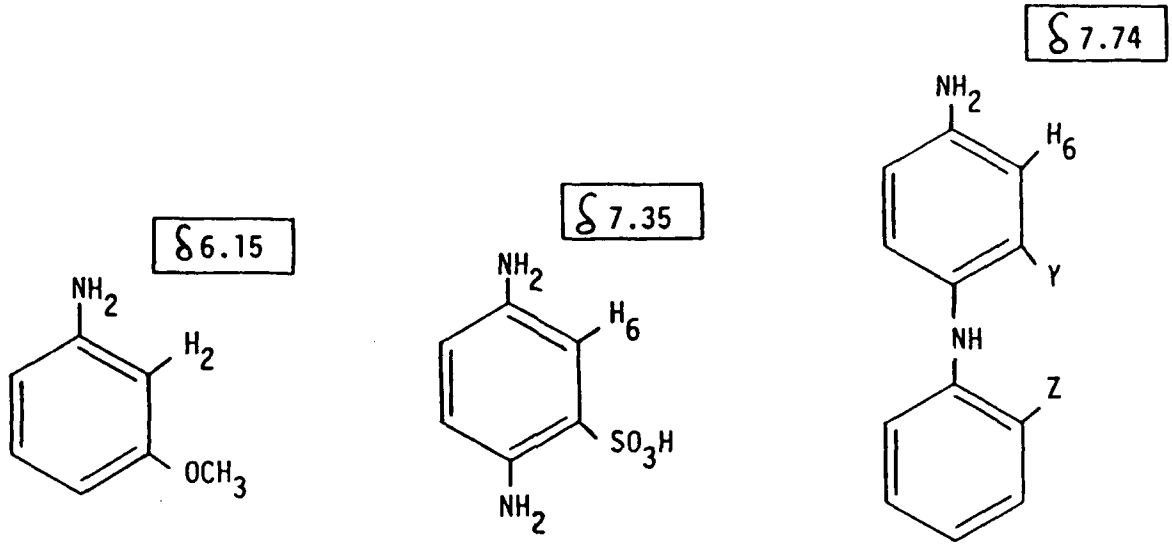
| Name | Reference |
|---|---|
| o-Ethoxy-o-Sulfo-p-Amino-Diphenylamine | CRLR 194[1] |
| o-Ethoxy-p-Amino-o'-Sulfodiphenylamine | CRLR 270[2] |
| p-Amino-o-Ethoxy-o-Sulfodiphenylamine | Purchase Description[8] |
| o-(4-Amino-o-phenetidino) Benzene Sulfonic Acid | CRLR 486[9] |
| p-Amino-o-Ethoxy-o'-Sulfo-Diphenylamine | Technical Manual[7]<br>Technical Manual[10]<br>Military Specification[4] |
| 4-Amino-2-Ethoxydiphenylamine-2-Sulfonic Acid | EASP 100-2[11] |
| o-Ethoxy-p-Amino-o-Sulfodiphenylamine | EASP 100-2[11] |
| p-Amino-o-Oxy-o'-Sulfo Diphenylamine (sic) | ARCSL-SP-79015[12] |

Table 2. Structures Reported for Compound 34

| Structure | Reference |
|---|---|
|  | EASP-100-2[11] |
|  | Technical Manual[10] |
|  | CRLR 194[1] |

In June 1976, a sample of Compound 34 was submitted by personnel from the Detection Directorate for NMR characterization. At that time, there was confusion over the positions of the substituents on the two rings. (This is certainly understandable based on all the discrepancies in the literature.) The sample was dissolved in deuterated dimethyl sulfoxide, and a 60 MHz $^1H$ NMR spectrum was obtained using a Varian A-60D NMR spectrometer. The spectrum

showed a resonance at delta 7.74 parts per million (ppm) for the isolated proton on the trisubstituted ring. Based on reference compounds [3-methoxyaniline (delta $H_2$ 6.15) and 2,5-diaminobenzenesulfonic acid (delta $H_6$ 7.35)], the conclusion was that the ethoxy was on the ring by itself and that the sulfonic acid moiety was on the ring with the primary amino group.




3-Methoxyaniline

2,5-Diaminobenzenesulfonic acid

Compound 34

In July 1978, Compound 34 was again characterized by NMR, this time using $^{13}C$. The $^{13}C$ NMR chemical shifts for the aromatic carbons were calculated using $^{13}C$ additivity rules.[5] One set of shifts was calculated assuming the ethoxy was on the same ring as the primary amino group, and a second set of shifts was calculated assuming the sulfonic acid moiety was on this ring (Table 3). The ranges for the $^{13}C$ NMR shift values for the aromatic carbons were 101.8-140.9 ppm and 114.0-149.2 ppm, respectively. The $^{13}C$ spectrum of Compound 34 obtained on a Varian FT-80A NMR spectrometer at 20 MHz showed a chemical shift range of 113.1-148.7 ppm for the aromatic carbon atoms (Table 3). The presence of the peak at delta 148.7 and the absence of peaks above 113 ppm indicates that the correct structure of Compound 34 is

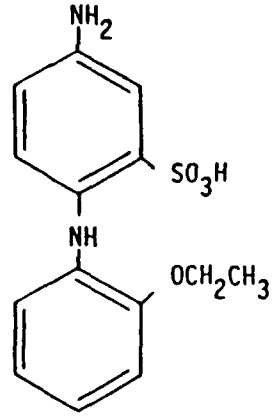
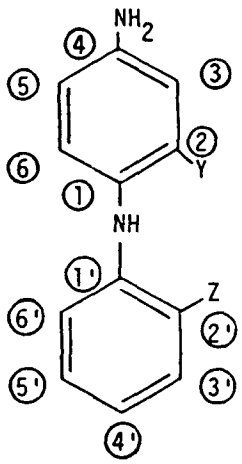


Table 3. Calculated and Experimental $^{13}C$ Chemical Shifts for the Aromatic Carbons of Compound 34 (July 1978)




| | Y=OEt, Z=$SO_3H$ | | Y=$SO_3H$, Z=OEt | Observed, DMSO-$d_6$ |
|---|---|---|---|---|
| 1 | 118.9 | 1 | 131.1 | 113.1 |
| 2 | 127.8 | 2 | 133.7 | 114.9 |
| 3 | 101.8 | 3 | 114.0 | 116.4 |
| 4 | 139.6 | 4 | 137.3 | 120.5 |
| 5 | 108.5 | 5 | 120.0 | 120.8 |
| 6 | 119.7 | 6 | 117.4 | 121.2 |
| 1' | 140.9 | 1' | 128.7 | 122.6 |
| 2' | 132.8 | 2' | 149.2 | 124.2 |
| 3' | 127.3 | 3' | 115.1 | 131.3 |
| 4' | 119.3 | 4' | 122.0 | 134.5 |
| 5' | 133.3 | 5' | 121.8 | 139.9 |
| 6' | 116.5 | 6' | 118.8 | 148.7 |
| Range of Deltas: | 101.8-140.9 | | 114.0-149.2 | 113.1-148.7 |

This was consistent with the $^1H$ NMR data obtained in 1976 and with the structure given in the Operator's Manual for the M19 Detector Kit.

Recent discussions with personnel in the Detection and Research Directorates showed that there were still many misconceptions regarding the structure and purity of the CRDEC lots of Compound 34. Consequently, a rigorous investigation was undertaken to determine the structure and purity of Compound 34. This report documents the results of this study.

## 3. EXPERIMENTAL SECTION

### 3.1 Materials.

The sample of Compound 34 (a purplish-blue solid) was obtained from the Detection Directorate, CRDEC, and was used "as received." The sample was

part of a batch of material that had been purchased from Allied Chemical and Dye Corporation (Buffalo, NY) in the mid-1950's. This batch of Compound 34 has been supplied to contractors for the past 25+ yr to make tablets to be used in the detector kits and is the only large batch of Compound 34 known to exist. Documentation on the synthesis and purchase of the compound could not be found.

Reactants and solvents for the syntheses, solvents for the thin-layer chromatography (TLC) study, as well as the deuterated dimethyl sulfoxide, DMSO-$d_6$ (MSD Isotopes), which was used as the solvent for the NMR studies, were all used "as received."

3.2 NMR Analyses.

The $^1H$ and $^{13}C$ NMR spectra were obtained using a Varian VXR-400s superconducting FTNMR system operating at 400 MHz for $^1H$ and 100 MHz for $^{13}C$. The spectra were obtained at probe temperature (+18 °C), and quantitative information was obtained via digital integration of the spectral regions of interest. The two-dimensional experiments (COSY, HETCOR, and NOESY) were performed using the standard Varian VNMR software.

3.3 MS Analyses.

Compound 34 and its acetylated derivatives were characterized by Direct Insertion Probe (DIP) MS in the electron ionization (EI) and chemical ionization (CI) modes. The spectra were obtained on a Finnigan Model 5100 GC/MS (San Jose, CA). The quartz tube probe was heated from 60 to 500 °C at 120 °C/min. The source temperature was 120 °C. The CI reagent was methane at a source pressure of 0.6 torr. The mass range was scanned from 40 to 450 amu for EI and from 60 to 450 amu for CI at a rate of 1 scan/s.

3.4 TLC Analysis.

The developing chamber used in this study was a glass cylindrical tank (2.5 in. by 9 in.). The chamber was prepared for use by introducing 20 mL of a chloroform/methanol (1:1) mixture along with a filter paper wick. The chamber was allowed to come to equilibrium for 45 min before introducing the chromatoplate.

The chromatoplate was prepared by cutting a 40 mm by 200 mm section from the 200 mm by 200 mm Eastman Chromatogram Sheet (Silica Gel without fluorescence). This section was scribed with a line horizontal to the base of the plate 100 mm above the point of sample application. The sample was applied 15 mm above the base of the plate. Additionally, three lines were scribed perpendicular to the base of the plate; one, 20 mm from the edge of the plate, and the other two, 2 mm from each edge. Thus, two individual chromatograms per sheet were obtained, each with minimal edge effects. Development was carried out at 24 ± 2 °C.

Compound 34 was dissolved in a methanol/water (80/20) mixture. The resulting blue solution was applied to the plate with a capillary tube drawn to a fine point. The methods of detection included visible light, ultraviolet (UV) light, iodine vapor, and palladium chloride solution. Areas were measured using a Desaga template.

3.5 Syntheses.

3.5.1 N-Acetyl-N'-(2'-Ethoxyphenyl)-1,4-Diaminobenzene (V).

Desulfonation of Compound 34 (600 mg) was affected by heating at 190 °C in 1:1 $H_2SO_4$/water (6 mL) for 6 hr. After cooling, water was added to give a final volume of 20 mL. The pH was adjusted to 8.5-9.0 by adding $Na_2CO_3$, and the amine was extracted into chloroform (3 by 15 mL). The chloroform solution was dried over $MgSO_4$, filtered, and then evaporated to yield a purple/blue oil that solidified on standing. The solid was extracted with 2N hydrochloric acid (HCl) (5.0 mL) and 5N HCl (5.0 mL). The acid fraction was added to a solution of sodium acetate (5.0 g in 25.0 mL water). Acetic anhydride (5.0 mL) was added, and the solution was shaken for 15 min. After standing for 1.5 hr, Structure V (80 mg) was filtered from the solution. Recrystallization from ethanol/water with decolorization using charcoal yielded a pale purple product [melting point uncorrected, 124.5-125.5 °C]. The $^{1}H$ and $^{13}C$ NMR data and the EI and CI mass spectral data are presented in Section 4.2.

3.5.2 N,N'-Diacetyl-5-Amino-2-(2'-Ethoxyphenyl)Aminobenzenesulfonic Acid (VI).

Compound 34 (500 mg) was refluxed in acetic anhydride (5.0 mL) for 15 min, then allowed to stand overnight (16 hr). Water (10.0 mL) was added to decompose any N,N-diacetamide present, and the solution was extracted with chloroform. The aqueous layer was freeze dried to yield a purple solid (ca. 500 mg). The $^{1}H$ and $^{13}C$ NMR data and the EI and CI mass spectral data are presented in Section 4.3.

4. RESULTS AND DISCUSSION

4.1 Compound 34.

4.1.1 Purity.

4.1.1.1 TLC Analysis.

The results of the TLC analysis of Compound 34 are shown in Table 4 and reveal the presence of one major component and two small impurities. One impurity, a very polar compound based on its $R_f$ value, represents no more than 6% of the total sample. This value is based strictly on the compound's area in the chromatogram when visualized with iodine vapor. The second impurity, which is the colored component of the Compound 34 sample, appears to be present only in a trace amount. Its presence was made evident in the chromatogram only by a very faint violet-colored spot. The impurity could not be visualized by either iodine vapor, UV light, or palladium chloride solution. Thus, based on the TLC analyses, the purity of the Compound 34 sample is 94-98%.

4.1.1.2 NMR Analysis.

The $^{1}H$ NMR spectrum of the CRDEC Lot of Compound 34 dissolved in deuterated dimethyl sulfoxide, DMSO-$d_6$, is shown in Figure 1a. No impurity peaks were observed, and the integration of the resonances was within experimental error (±3%). Therefore, this sample of Compound 34 is >95% pure by NMR.

Table 4. TLC Results for Compound 34

| Spot Number | $R_f$ Value | Area ($mm^2$) | Visible Light | UV Light | Iodine Vapor | Paladium Chloride |
|---|---|---|---|---|---|---|
| 1 | 0.00 | 6 | - | Blue-White | Reddish-Brown | Reddish-Brown |
| 2 | 0.63 | 95 | - | Blue-White | Reddish-Brown | Reddish-Brown |
| 3 | 0.78 | (3) | Faint-Violet | | | |

4.1.2 NMR Characterization.

4.1.2.1 $^1H$ NMR.

Integration of the $^1H$ spectrum (Figure 1b) revealed that Compound 34 contains seven aromatic protons and one ethoxy group ($CH_3$: delta 1.33, triplet, J=7.0 Hz; $OCH_2$: delta 4.01, quartet, J=7.0 Hz). In addition, three resonances corresponding to slowly exchanging protons were present, one (delta 3.43) of which arises from the water in the solvent. Integration of the other resonances indicated that one corresponds to a single proton (delta 8.66) and was assigned as the proton on the secondary amine; whereas, the number of protons represented by the other resonance (delta 9.69) varied from 2 to 3 between solutions. Presumably, this corresponds to the primary amino group exhibiting a variable degree of protonation by the sulfonic acid moiety. Thus, the gross detail of the $^1H$ NMR spectrum was consistent with two of the proposed structures of Compound 34 (Table 2) and showed that the CRDEC sample was not a sodium salt. (A $^{23}Na$ NMR of the DMSO-$d_6$ solution of Compound 34 showed no resonances, further confirming that the sample was not a sodium salt).

The $^1H$ COSY spectrum was used to provide the through-bond connectivities of the aromatic $^1H$ nuclei of the sample (Figure 2). Two isolated aromatic spin systems containing three and four protons were readily identified. Analysis of the three proton spin system provided Substructure I. Thus, $H_6$ is meta to a single hydrogen, $H_4$ ($J_{H6-H4}$ = 2.6 Hz), which in turn is ortho to $H_3$ ($J_{H4-H3}$ = 8.7 Hz). The second aromatic ring (the four proton spin system) was easily identified as having Structure II. The two resonances of this spin system that were resolved are not coupled to each other but do have more than one coupling. Thus, the substituents on this ring are ortho.

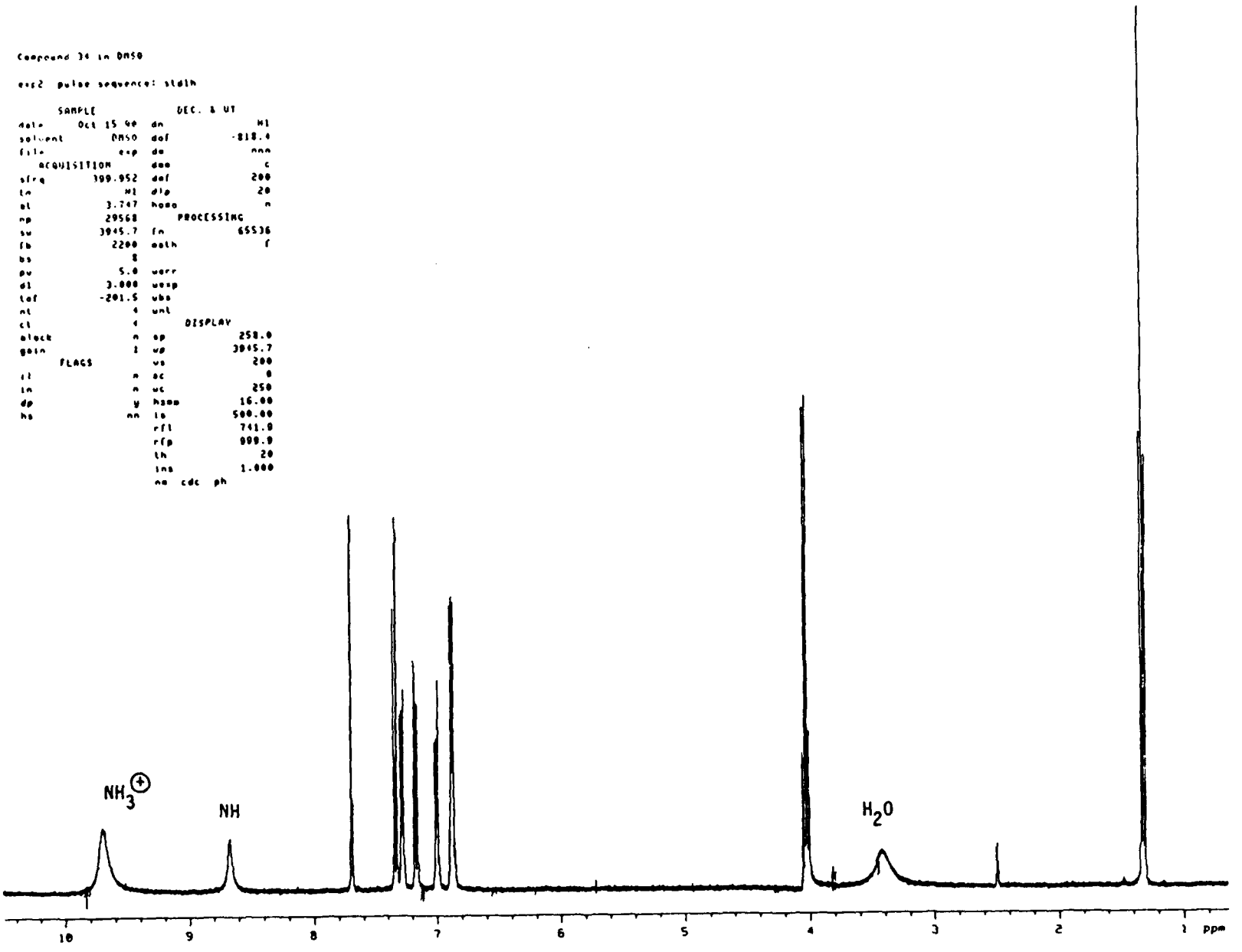



Figure 1a. Compound 34: $^{1}H$ NMR Spectrum in DMSO-$d_6$

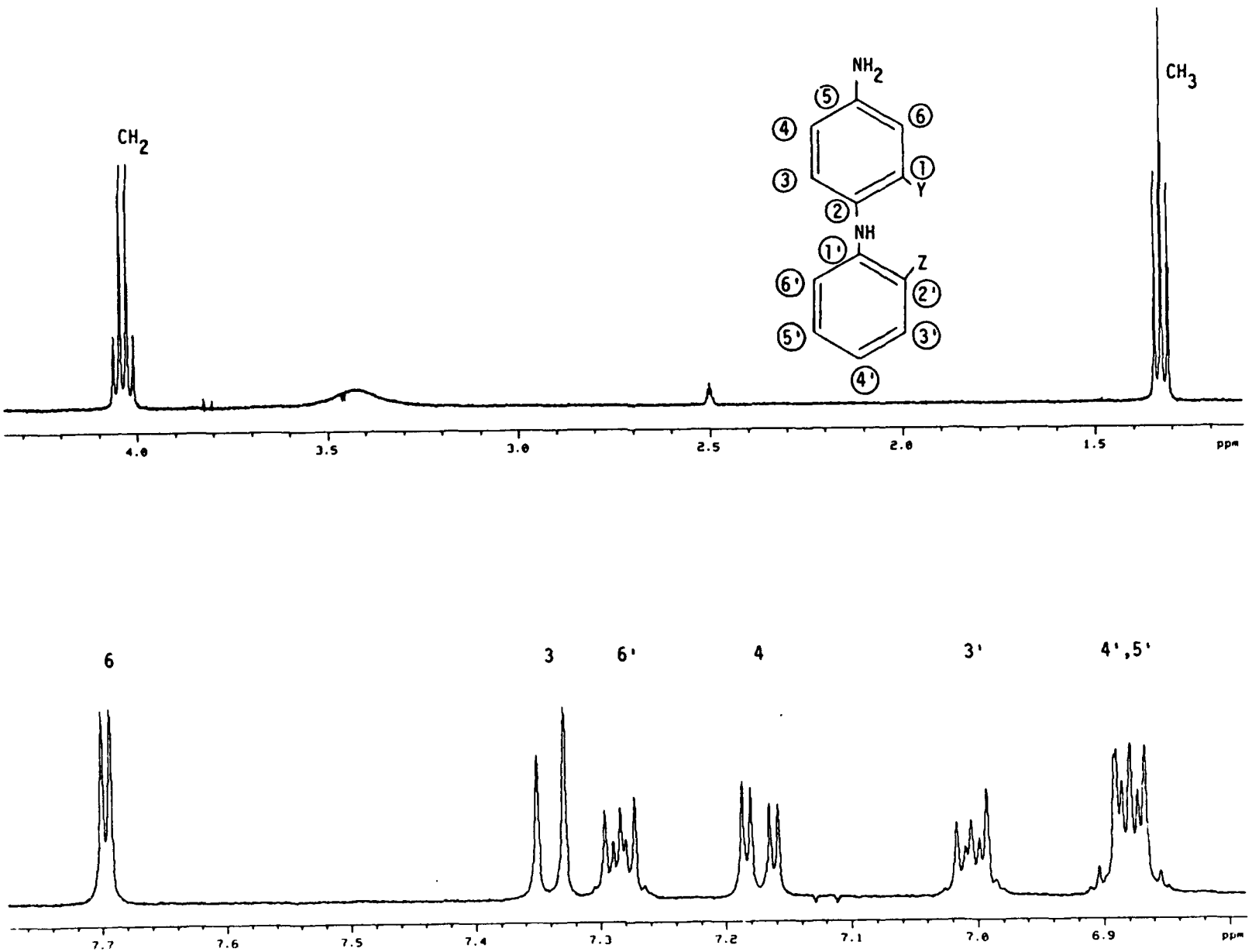



Figure 1b. Compound 34: Expanded $^{1}H$ NMR Spectrum

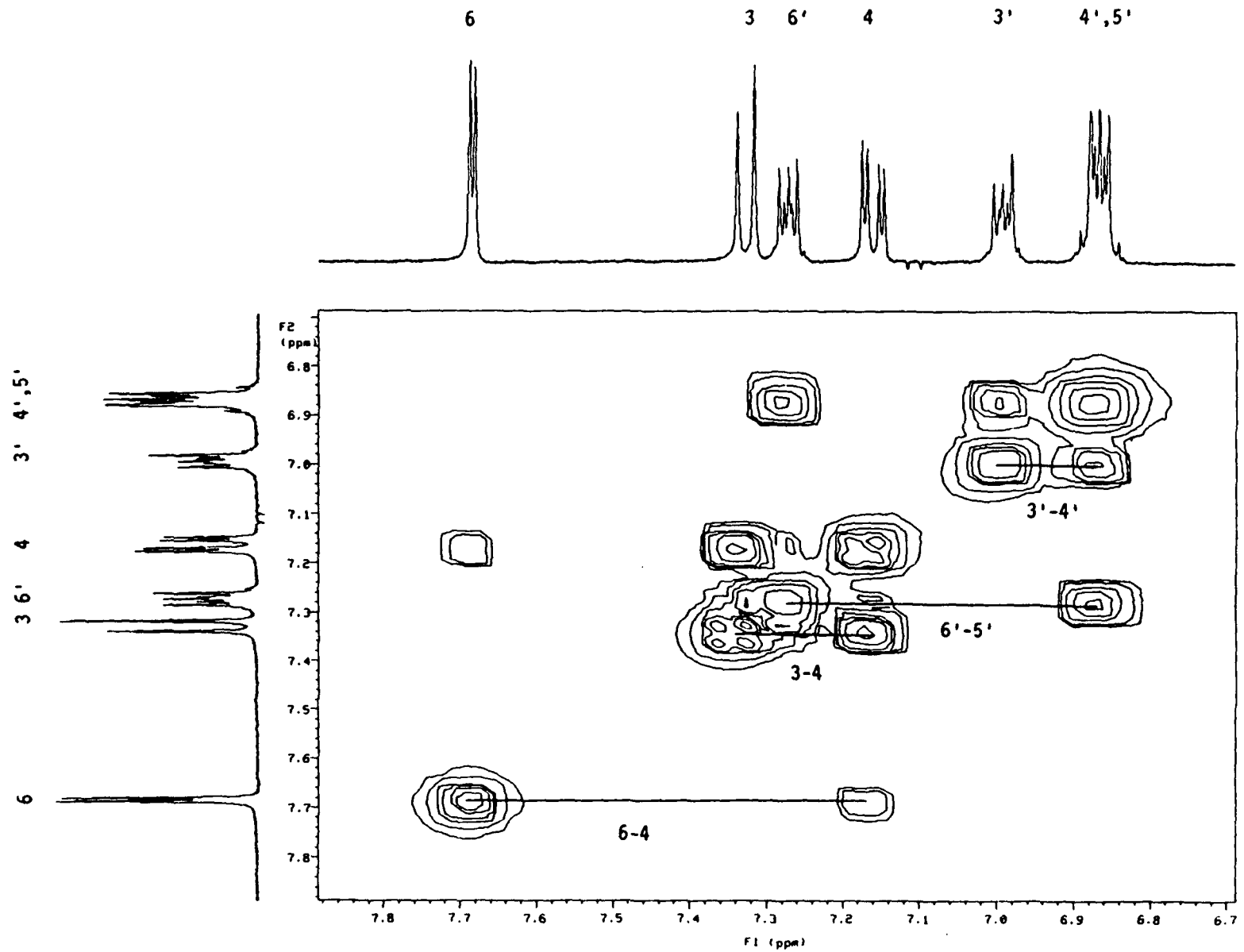
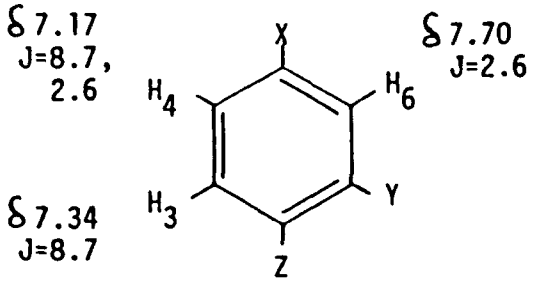



Figure 2. Compound 34: $^{1}H$ COSY Spectrum, Aromatic Region

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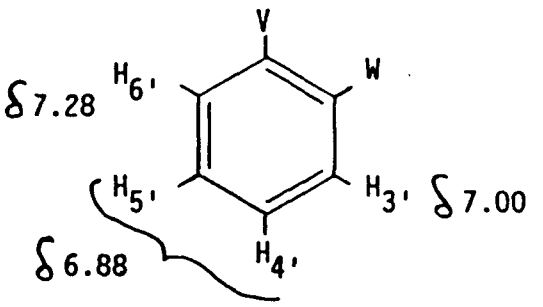
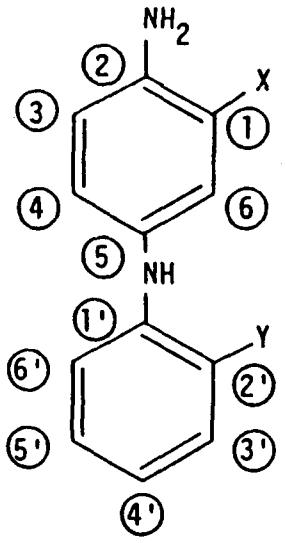
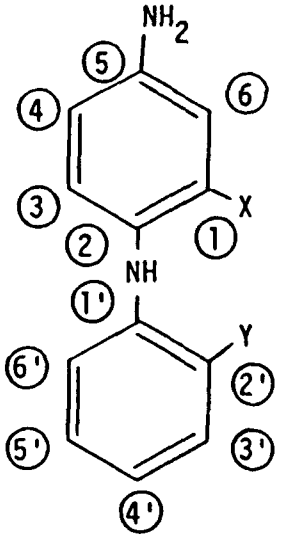



II

Two arrangements are possible when combining Substructures I and II to give the skeleton of Compound 34. A consistent feature of all the names is that each indicates the presence of the N-phenyl-para-phenylenediamine moiety. Although not yet proven, this structural feature is presumed correct. Substructures I and II are therefore combined to give the possible structures of IIIa and IIIb. A homonuclear NOE (nuclear Overhauser enhancement) experiment was used to determine a through-space connection between the $CH_2$ of the ethoxy group and one of the aromatic spin systems (Figure 3). Irradiation of delta 4.01 induced an enhancement of the methyl resonance (delta 1.33) and the aromatic resonance at delta 7.00, previously assigned as $H_{3'}$. Thus, the identity of substituent Y in IIIa and IIIb is established as ethoxy.




IIIa




IIIb

4.1.2.2 $^{13}C$ NMR.

The $^{13}C$ NMR spectrum and the $^{1}H$-$^{13}C$ 2D HETCOR spectra are consistent with both structures proposed by analysis of the $^{1}H$ spectra. The $^{13}C$ spectrum (Figure 4) contains resonances for each chemically distinct carbon in Compound 34; one methyl (delta 14.7) and one methylene (delta 64.0) for the ethoxy group, seven aromatic methines, and five aromatic quaternary carbons. The number of directly bonded hydrogens on each carbon was determined using the Attached Proton Test (APT) experiment. The directly coupled HETCOR experiment

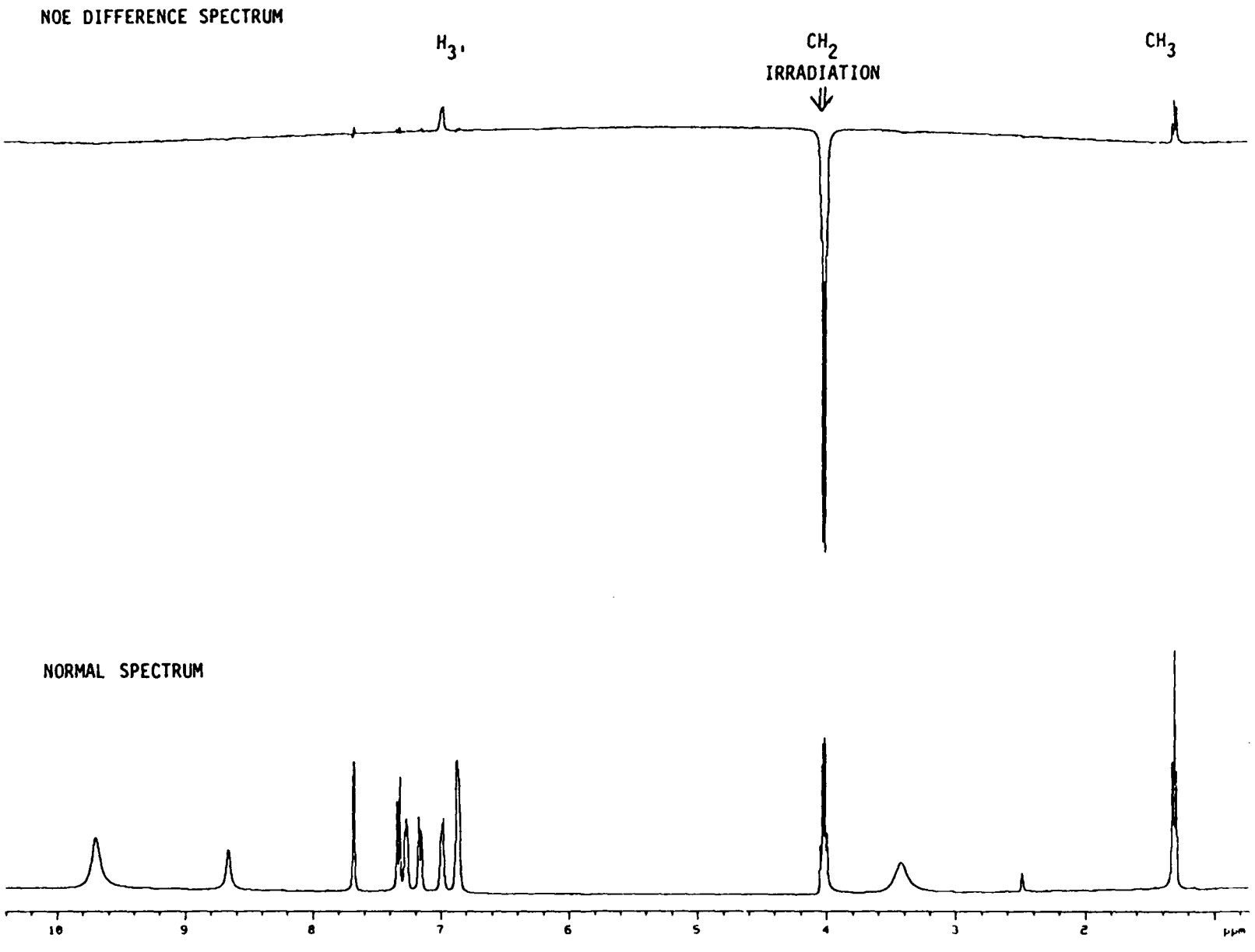



Figure 3. Compound 34: 1-D NOE Experiment, Irradiation of $CH_2$

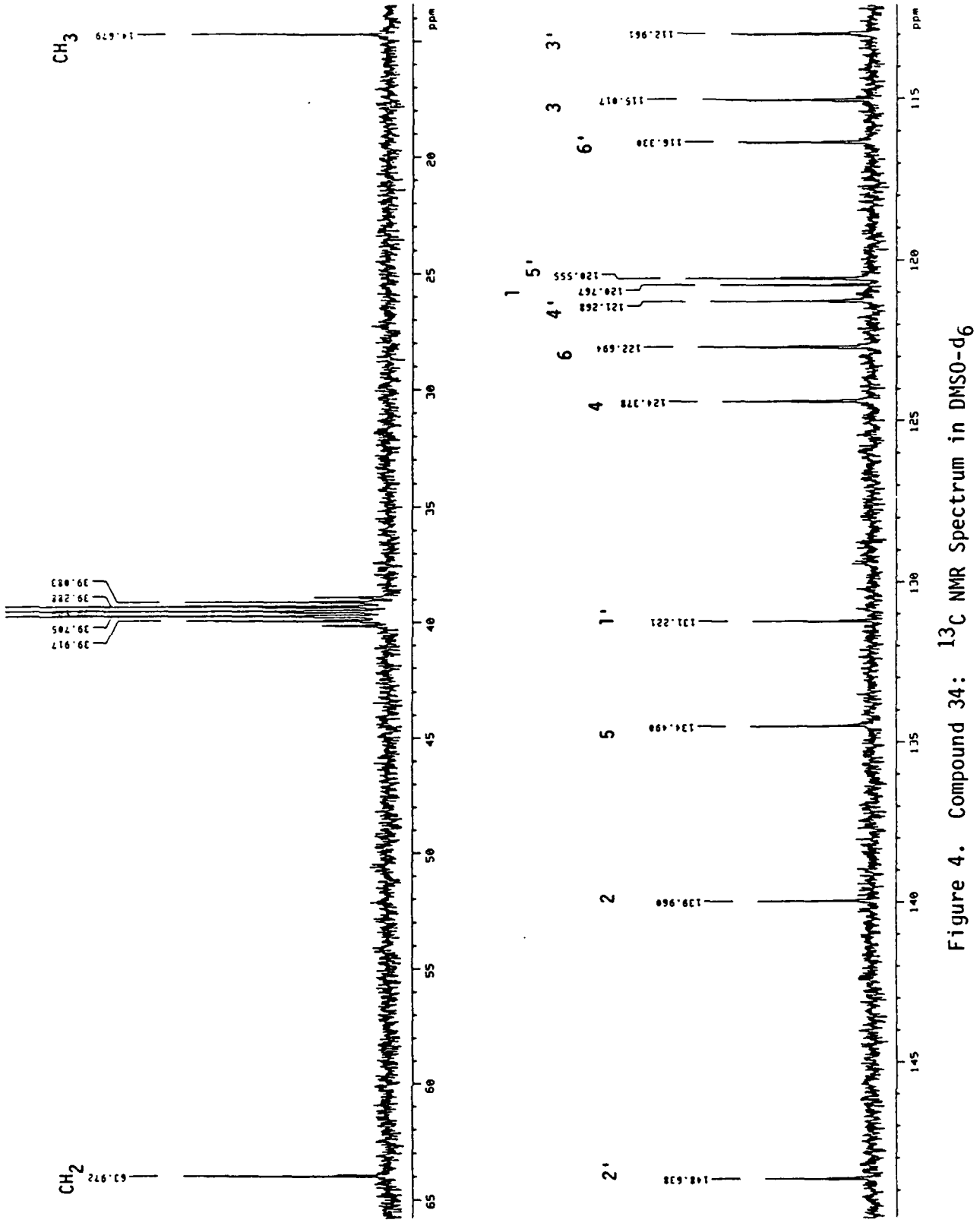
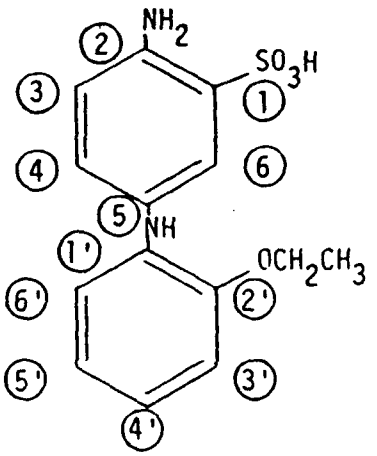
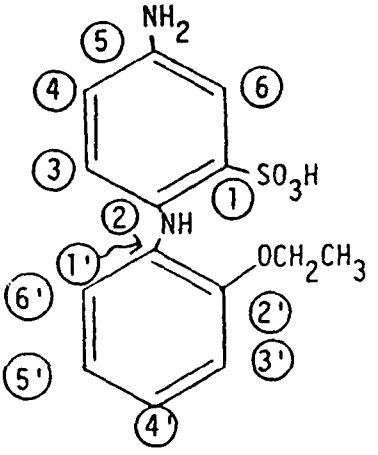



Figure 4. Compound 34: $^{13}C$ NMR Spectrum in DMSO-$d_6$

(Figure 5, Table 5) allows facile assignment of all but two of the aromatic methine resonances (deltas 120.6 and 121.3) to Structures IVa and IVb. The remaining methine resonances and those of the quaternary carbons are assigned from the long-range HETCOR experiment (Figure 6a, 6b, and Table 5). From the previously assigned methine resonances, the strongest long-range correlations appear to correspond to couplings with protons on meta carbons. Thus, the resonances at deltas 122.7, 124.4, and 113.0 exhibit strong correlations to their meta protons. In addition, much less intense correlations with ortho protons are evident for $C_3$ and $C_4$. $C_6$ shows an even less intense correlation with its para-hydrogen ($H_3$). The $^{13}C$ resonances at deltas 120.6 and 121.3 have a single, strong correlation with $H_{3'}$ and $H_{6'}$, respectively. These are assumed to arise from correlations with meta hydrogens, and allow unambiguous assignment of the resonances to $C_{5'}$ (delta 120.6) and $C_{4'}$ (delta 121.3).




IVa




IVb

The only aromatic methine resonance not exhibiting a long-range C-H correlation is that at delta 116.3 ($C_{6'}$). In the $^1H$-coupled $^{13}C$ spectrum, the lines of the corresponding doublet are the broadest and reflect the presence of a number of unresolved long-range couplings. The intensity of the resonance for $C_{6'}$ in the long-range HETCOR experiment will therefore be divided among a number of correlations that, although present, do not exceed the noise level and are not detected. If the $^1H$ signal has a large number of homonuclear couplings,[6] the intensity of correlations to individual proton resonances is also diminished. In this instance, the missing correlation is to $H_{4'}$, which is coupled to three other $^1H$ nuclei.

Working from the observation that the strongest correlations correspond to couplings with meta hydrogens, assignment of the quaternary carbon resonances is straightforward (Table 5). The strong (meta) correlations of the resonances at deltas 131.2, 140.0, and 148.6 provide for their unequivocal assignment as $C_{1'}$, $C_2$, and $C_{2'}$, respectively. Note that the meta correlations to $H_{4'}$ and $H_{5'}$ are weaker than expected and that the correlation between $C_{2'}$ and $H_{4'}$ is below the noise level. The low intensity of correlations to extensively coupled $^1H$ nuclei was discussed in the above paragraph.

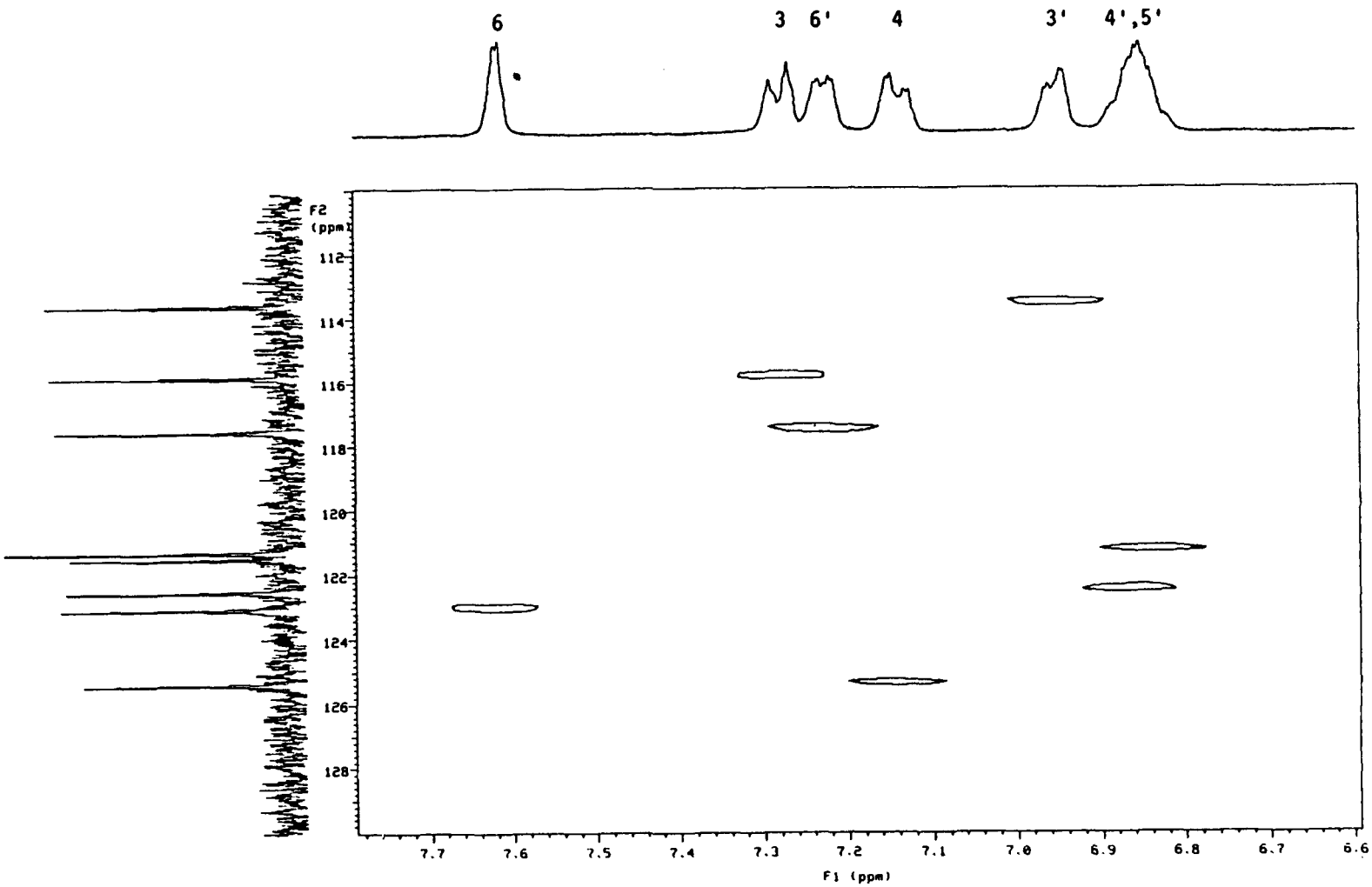



Figure 5. Compound 34: Directly Coupled HETCOR Experiment, Aromatic Region

Table 5. $^{1}H$-$^{13}C$ Correlations for the Aromatic Resonances in Compound 34

| Delta $^{13}C$, ppm | Directly Coupled Correlations | Long-Range Correlations | Assignment |
|---|---|---|---|
| 113.0 (d) | 7.00 ($H_3'$) | 6.88 (s, $H_4'$ or $H_5'$) | $C_3'$ |
| 115.0 (d) | 7.34 ($H_3$) | 7.17 (w. $H_4$) | $C_3$ |
| 116.3 (d) | 7.28 ($H_6'$) | - | $C_6'$ |
| 120.6 (d) | 6.88 ($H_4'$ or $H_5'$) | 7.00 (s, $H_3'$) | $C_5'$ |
| 121.3 (d) | 6.88 ($H_4'$ or $H_5'$) | 7.28 (s, $H_6'$) | $C_4'$ |
| 122.7 (d) | 7.70 ($H_6$) | 7.17 (s, $H_4$)<br>7.34 (v.w., $H_3$) | $C_6$ |
| 124.4 (d) | 7.17 ($H_4$) | 7.70 (s, $H_6$)<br>7.34 (w, $H_3$) | $C_4$ |
| 120.8 (quat) | - | 7.34 (s, $H_3$)<br>7.70 (w, $H_6$) | $C_1$ |
| 131.2 (quat) | - | 7.00 (s, $H_3'$)<br>7.28 (w, $H_6'$)<br>6.88 (w, $H_4'$ or $H_5'$) | $C_1'$ |
| 134.5 (quat) | - | 7.34 (s, $H_3$)<br>7.70 (w, $H_6$)<br>7.17 (w, $H_4$) | $C_5$ |
| 140.0 (quat) | - | 7.17 (s, $H_4$)<br>7.70 (s, $H_6$) | $C_2$ |
| 148.6 (quat) | - | 7.28 (s, $H_6'$)<br>7.00 (w, H ) | $C_2'$ |

d = doublet; quat = quaternary carbon; v.w. = very weak; w = weak; s = strong

The unassigned resonances (deltas 120.8 and 134.5) must correspond to $C_1$ and $C_5$, both of which should and do display a strong (meta) correlation with $H_3$ and a weak (ortho) correlation with $H_6$. The delta 134.5 resonance also has a weak (presumably ortho) correlation with $H_4$ and is therefore assigned as $C_5$. Thus, the resonance at delta 120.8 is that of $C_1$. These assignments may be reversed but would require that the para-correlation between $C_1$ and $H_4$ be stronger than the ortho correlation between $C_5$ and $H_4$. The only para-correlation observed in the experiment ($C_6$ to $H_3$) is much less intense than the ortho correlations.

4.1.2.3 $^{13}C$ Chemical Shifts Calculated from Substituent Effects.

The connectivities between nuclei extracted from the various $^{1}H$ and $^{13}C$ NMR experiments cannot distinguish between IVa and IVb as the correct structure for Compound 34. Prior to the development of 2-D NMR techniques, structure determination using NMR relied heavily upon empirical correlations between chemical shifts and environment for both $^{1}H$ and $^{13}C$ nuclei. The information available from chemical shifts is valuable and should not be neglected. Approximate $^{13}C$ chemical shifts can be calculated by combining the shifts of suitable model compounds with the effect of structural modification upon chemical shifts. The most appropriate model for Compound 34 is N-phenyl-p-phenylenediamine, which is shown below with its $^{13}C$ chemical shifts.[7]

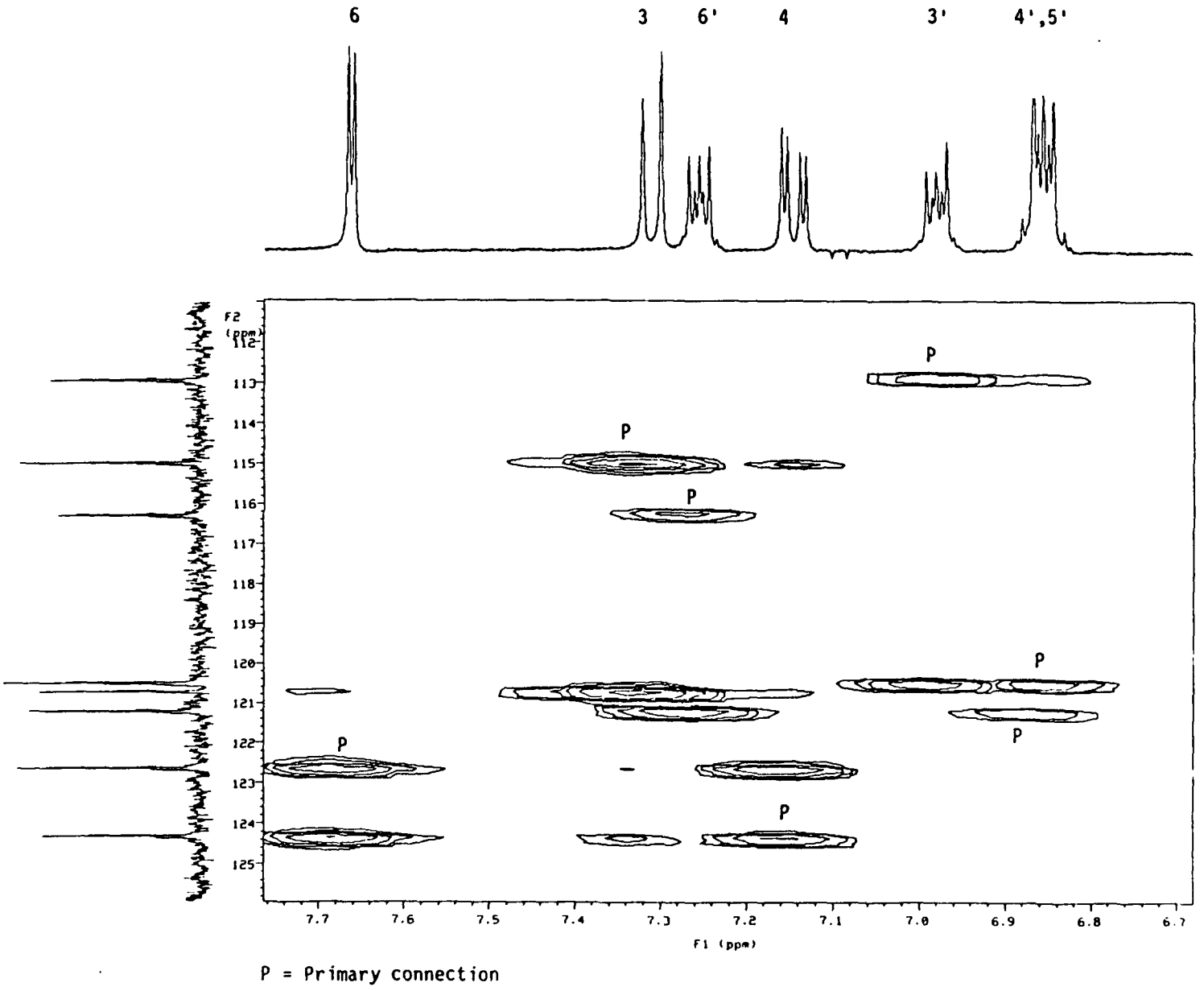



Figure 6a. Compound 34: Long-Range HETCOR Experiment, Aromatic Region

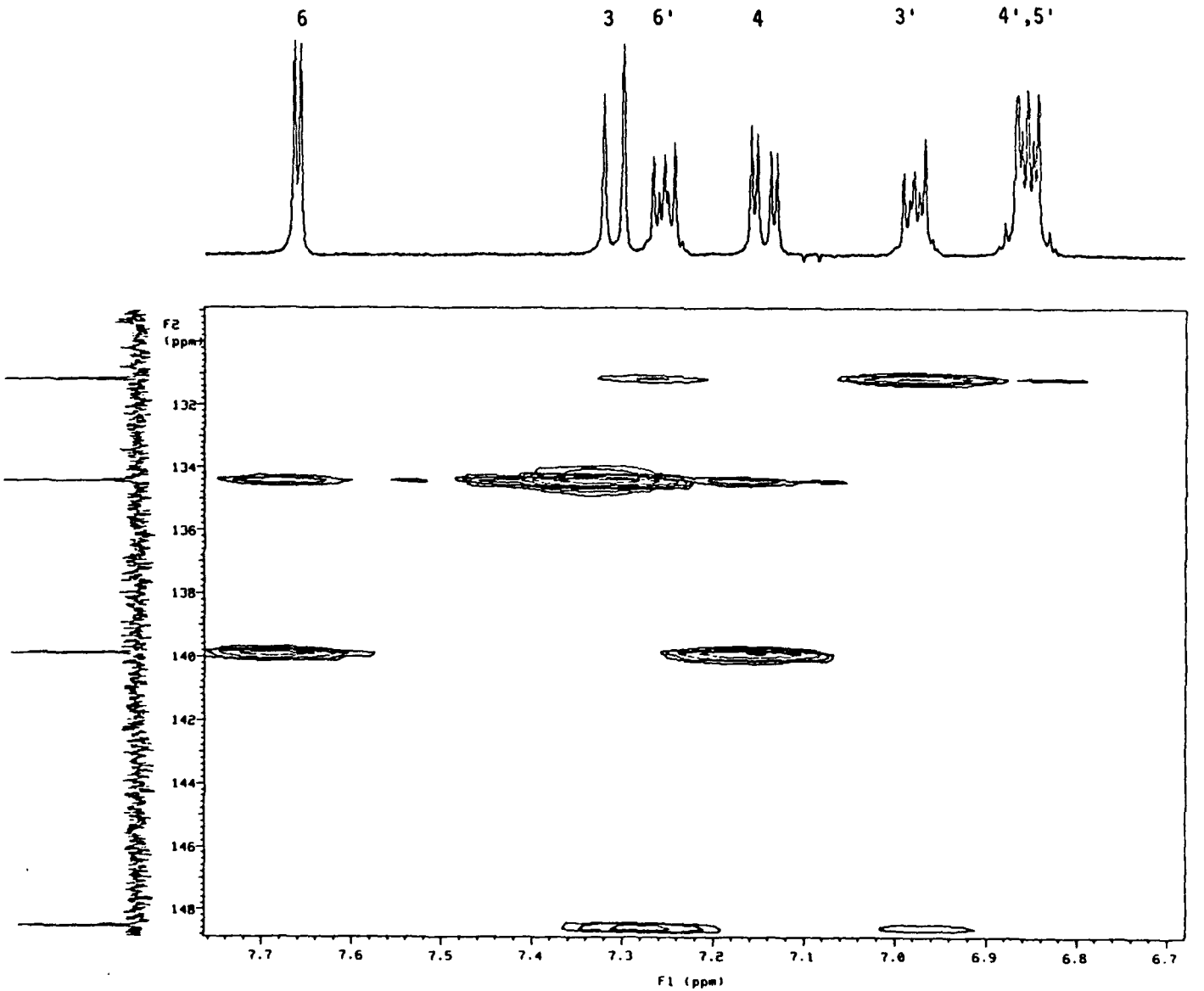
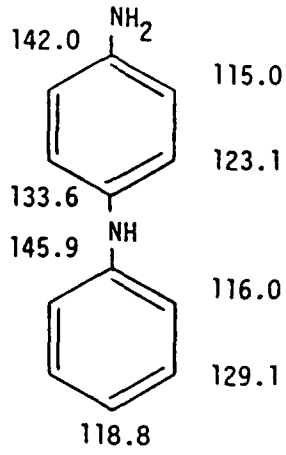
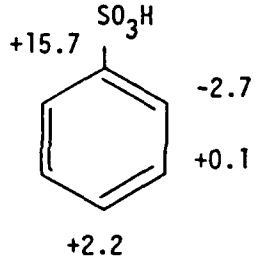


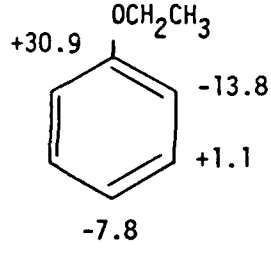

Figure 6b. Compound 34: Long-Range HETCOR, Aromatic Region



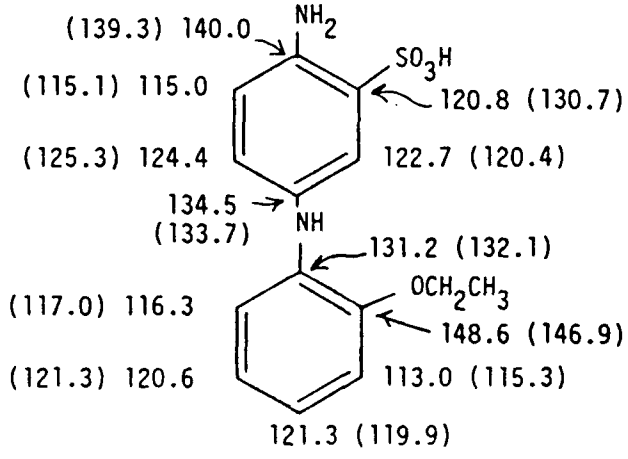

N-Phenyl-p-Phenylenediamine

The effects that substitution of aromatic hydrogens by the sulfonic acid and ethoxy moieties have on the $^{13}C$ shifts of the aromatic carbons (i.e., the substituent effects) are shown below.[7]

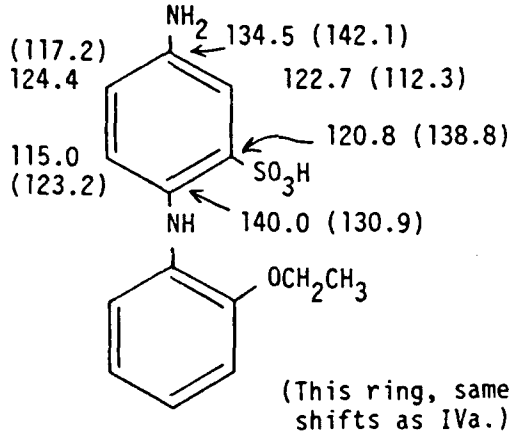



The chemical shifts calculated for IVa and IVb from the reported shifts for N-phenyl-para-phenylenediamine and these substituent effects are compared with the experimental values below. (Calculated values are in parentheses).




IVa




IVb

Excellent agreement between the calculated and experimental shifts is obtained for the ethoxy substituted ring, which is the same in both structures. For the trisubstituted ring, the agreement is much better for IVa than for IVb. The only significant difference between the calculated and experimental shifts in IVa is for $C_1$. Therefore, arguments based on chemical shifts alone indicate that IVa is the correct structure for Compound 34. This structural assignment is not definitive as the substituent effects used in the calculations are for monosubstituted benzenes, and do not reflect the influence that steric and electronic interactions between substituents may exert. Furthermore, the very large discrepancy between the calculated and experimental shifts for $C_1$ (i.e., sulfonic acid substituted carbon) in both IVa and IVb suggests that the substituent constant used for the sulfonic acid group may not be appropriate for this compound. Additional discussion regarding substituent effect calculations for IVa and IVb is included in Section 4.3.1.

4.1.2.4 $^1H$ Homonuclear NOE.

Differentiation between IVa and IVb as the correct structure for Compound 34 may be achieved by establishing the through-space relationship of the $NH_2$ protons with the aromatic protons of the phenylenediamine ring. In theory, a homonuclear NOE experiment with irradiation of the $NH_2$ protons (Figure 7) should provide this relationship. In practice, interpretation of this experiment is ambiguous. Irradiation of the $NH_2$ resonance also causes saturation of the secondary amine proton and water resonances, indicating exchange of protons between the three environments. Thus, although two resonances ($H_4$ and $H_6$) are enhanced by irradiation of the $NH_2$, indicating IVb as the correct structure, it is possible that the interaction is with either the $NH_2$ or the NH protons. In order to obtain the through-space relationship between the $NH_2$ and the aromatic ring, the $NH_2$ group was acetylated. Amide protons do not exchange, and an NOE experiment with irradiation of the amide proton would provide the required information (Section 4.3.1).

4.1.3 MS Characterization.

The EI and CI mass spectra of Compound 34 were obtained and are shown in Figure 8. The spectra indicate that desulfonation occurs under MS conditions. The EI base peak at m/z 228 corresponds to the molecular weight of the desulfonated product, and the ions at m/z 64 and 48 represent the spectrum of $SO_2$. Similarly, the CI base peak at m/z 229 corresponds to the protonated molecular ion of the desulfonated product, and the ion at m/z 65 represents the protonated molecular ion of $SO_2$. Structural assignments for the CI fragmentation products are shown in Table 6.

4.2 Desulfonated/Acetylated Compound 34 (V).

To confirm the earlier homonuclear NOE experiment (Section 4.1.2.4) that indicated that the ethoxy group was on the ring by itself (Structure III, Y=ethoxy), Compound 34 was desulfonated. This desulfonated product was acetylated to obtain a pure compound (V) that was identified by its $^1H$ and $^{13}C$ NMR spectra and its EI and CI mass spectra.

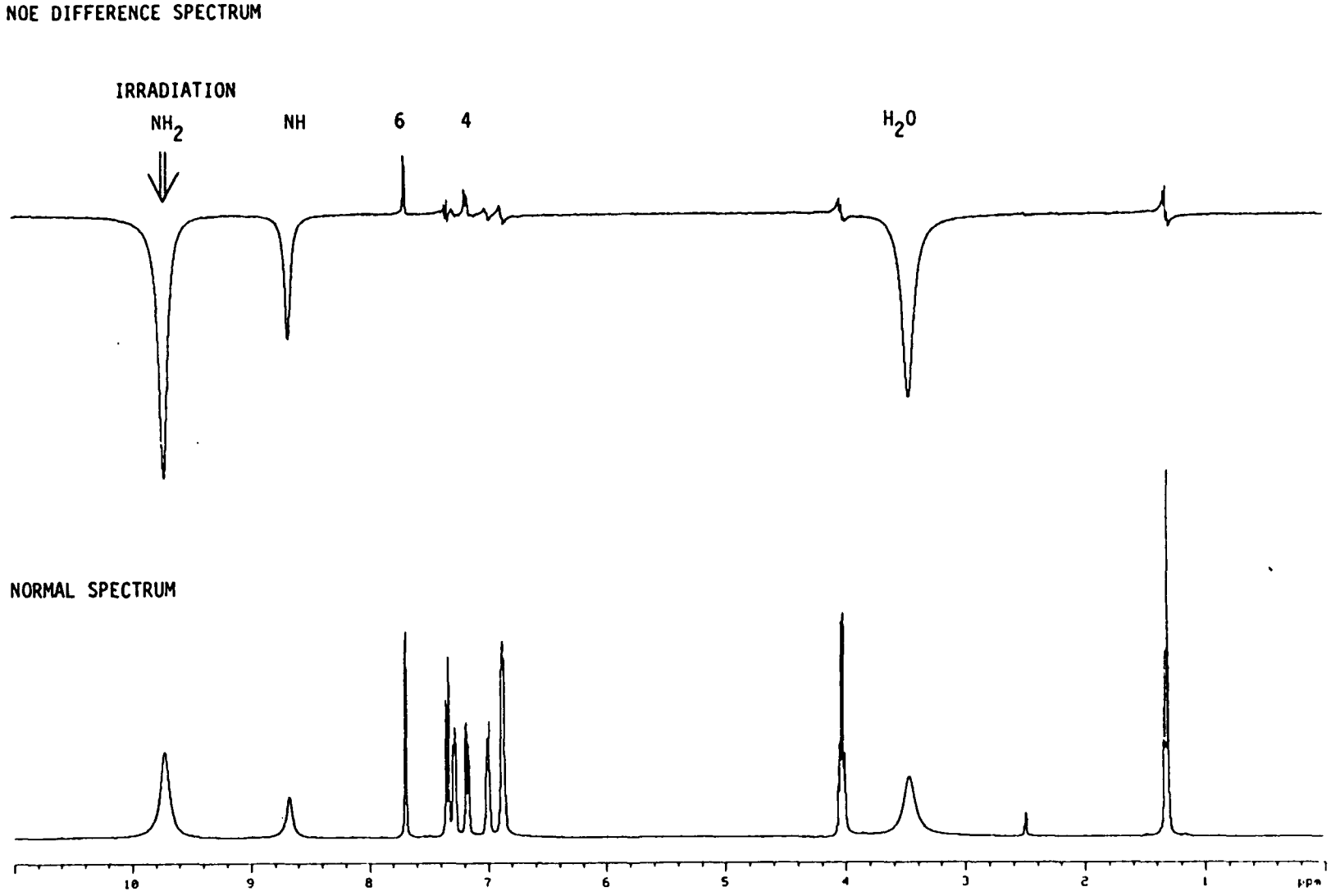



Figure 7. Compound 34: 1-D NOE Experiment, Irradiation of $NH_2$

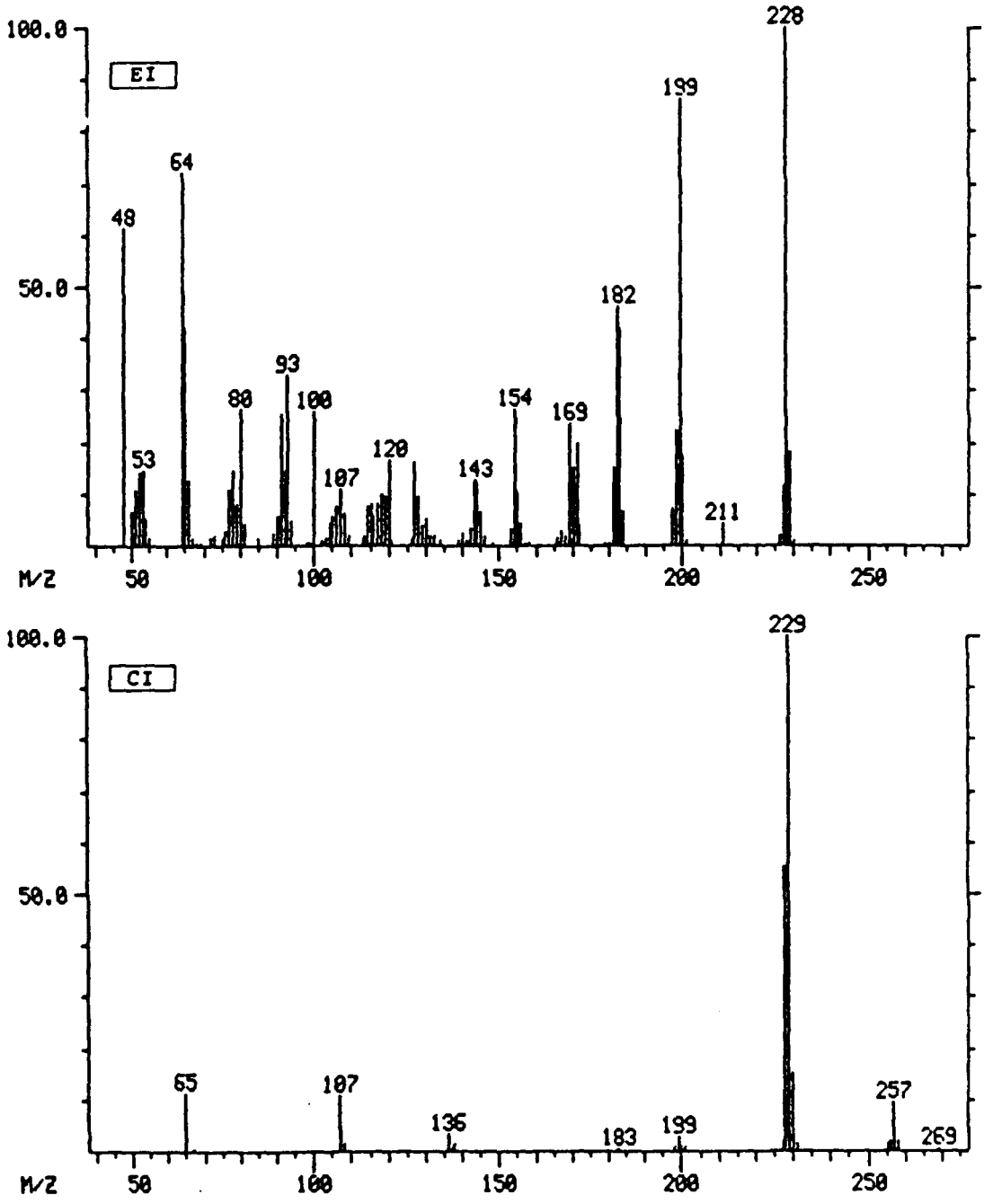
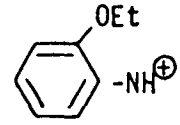
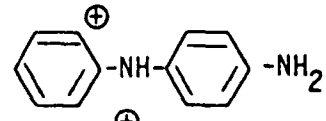
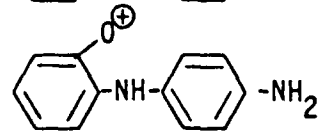
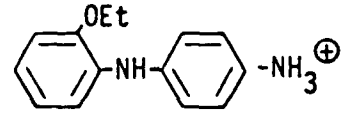
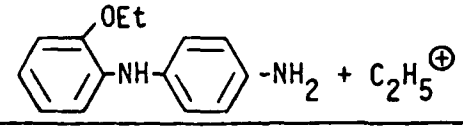
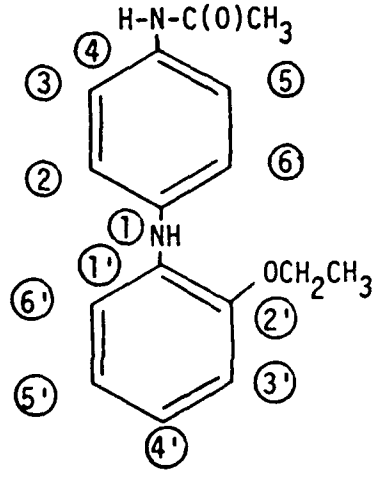



Figure 8. Compound 34: EI and CI Mass Spectra

Table 6. Compound 34 CI Mass Spectral Fragmentation Ions

| M/Z | Structural Assignment | Relative Intensity |
|---|---|---|
| 65 | $SO_2H^{\oplus}$ | 12 |
| 107 |  | 12 |
| 136 |  | 3 |
| 183 |  | 1 |
| 199 |  | 3 |
| 229 |  | 100 |
| 257 |  | 10 |



V

4.2.1 NMR Characterization.

Unambiguous identification of the structure of V is possible from its $^{1}H$ and $^{13}C$ NMR spectra (Figures 9a, 9b, and 10). Two four-spin systems in the aromatic region of the $^{1}H$ spectrum can be identified in the $^{1}H$ COSY spectrum

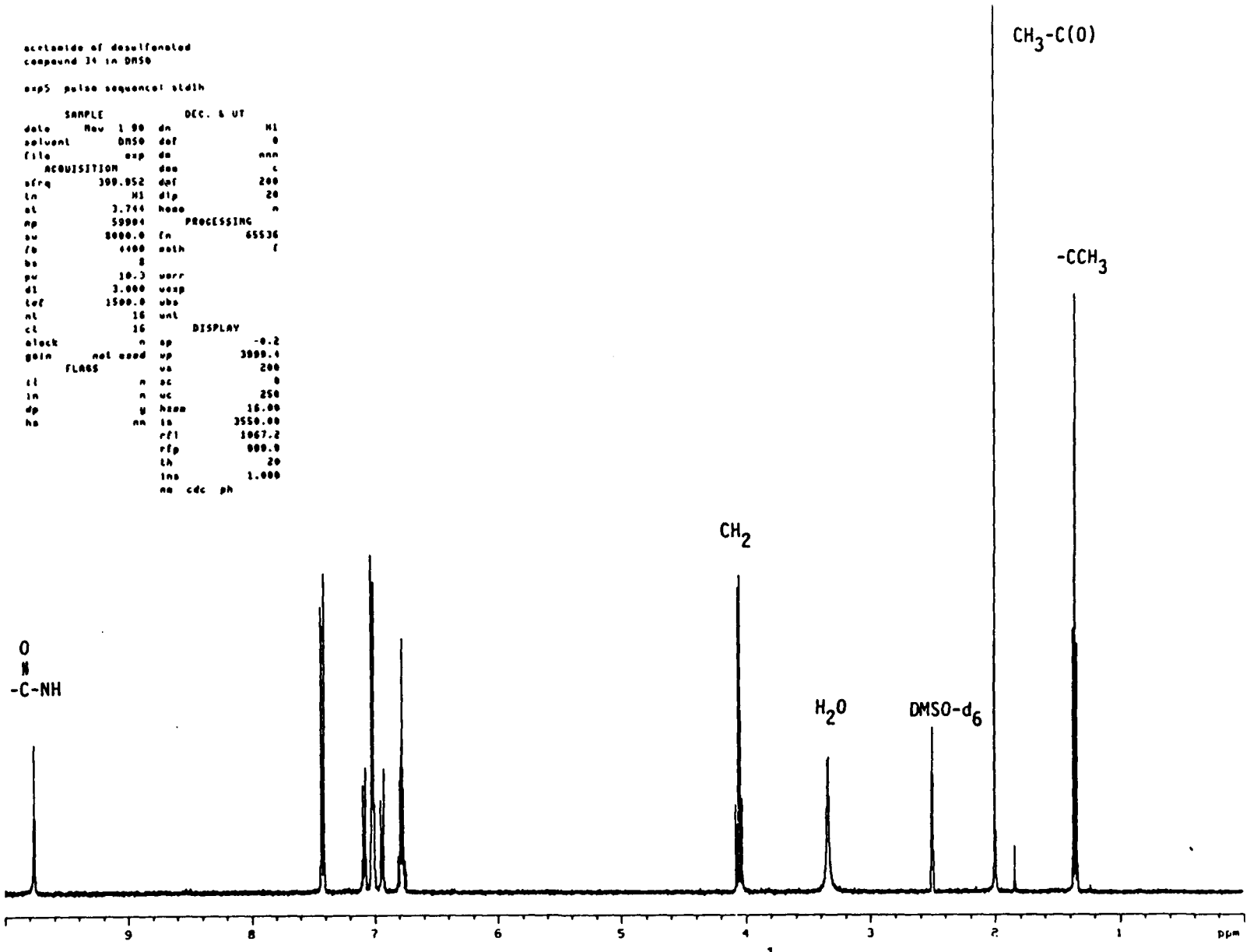



Figure 9a. Desulfonated/Acetylated Compound 34 (V): $^{1}H$ NMR Spectrum in DMSO-$d_6$

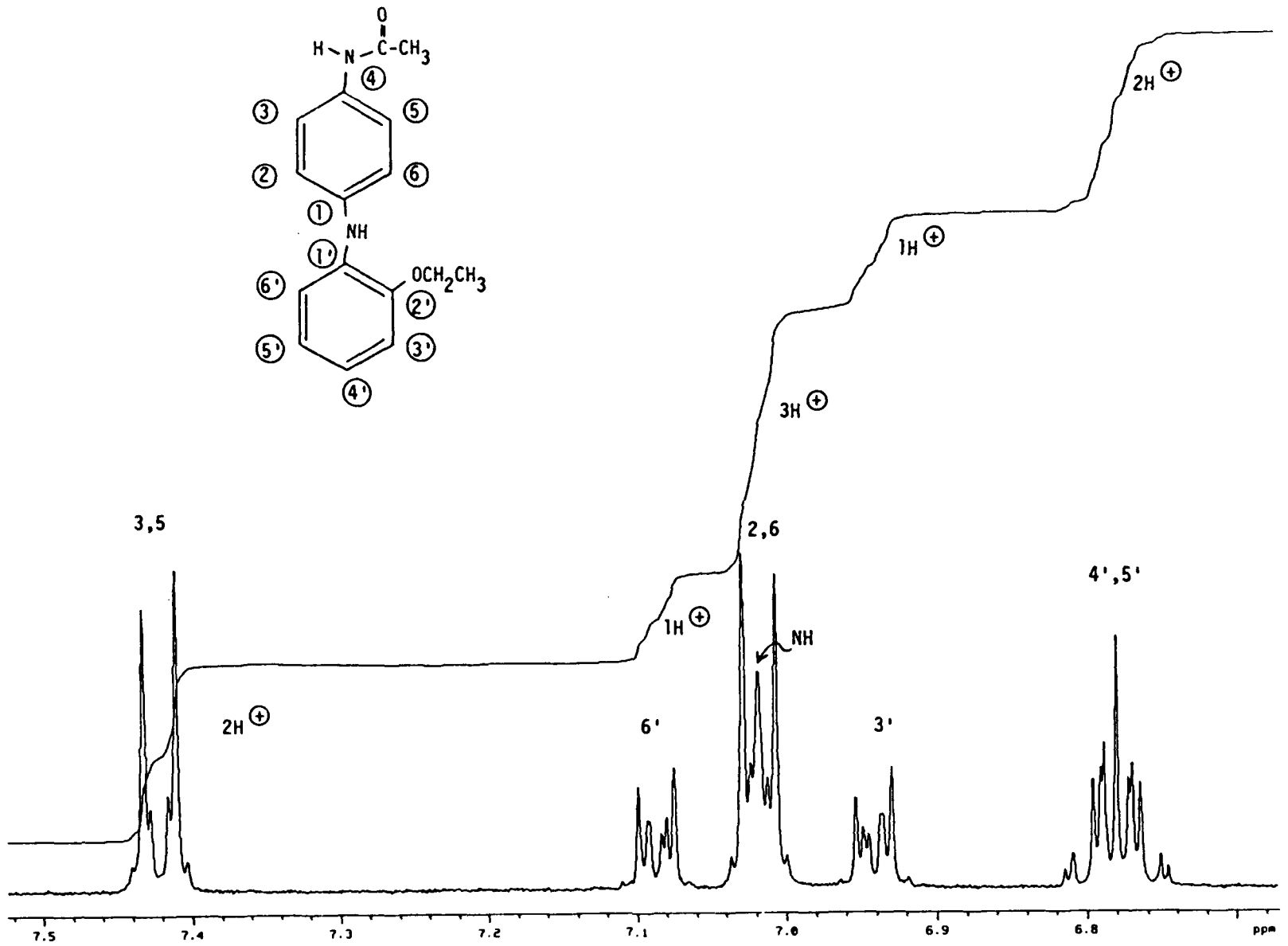



Figure 9b. Desulfonated/Acetylated Compound 34 (V): Expanded $^{1}H$ Spectrum, Aromatic Region

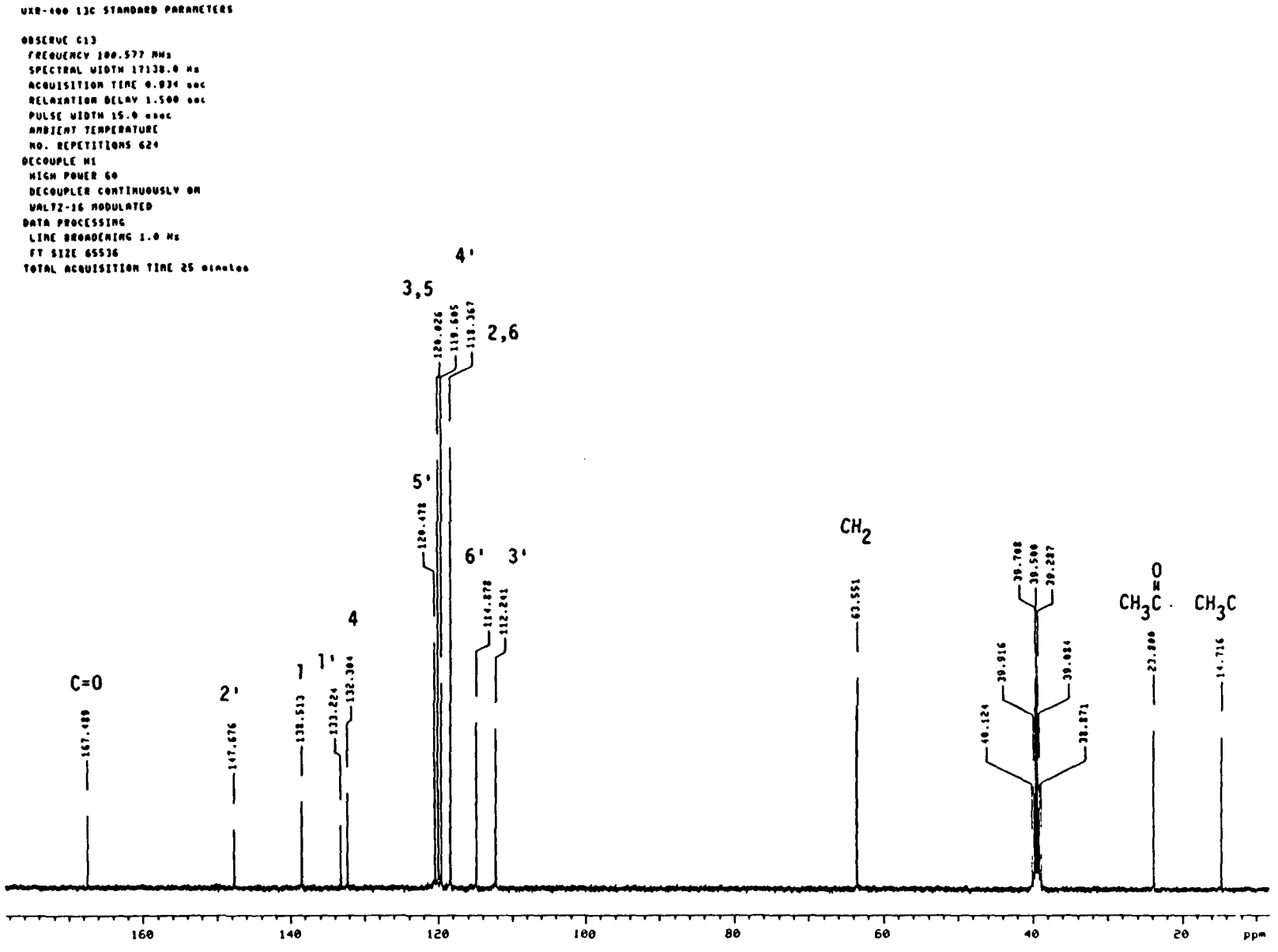



Figure 10. Desulfonated/Acetylated Compound 34 (V): $^{13}C$ NMR Spectrum in DMSO-$d_6$

(Figure 11). The para- and ortho-substitution patterns indicated for the two aromatic rings in Structure V are confirmed by analyzing the couplings within each of the spin systems. Thus, both the position of the ethoxy group on the ring by itself and the assumed para-relationship of the amino groups in the phenylenediamine moiety of Compound 34 are firmly established. Resonances corresponding to hydrogens in the ethoxy substituted ring were readily assigned by comparison with the spectrum of Compound 34. Assignment of the $^{1}H$ shifts for the para-substituted ring, and the remaining resonances (i.e., ethoxy, methyl, amino, and amide protons) were based upon chemical shifts (Table 7). Confirmation of the assignments for all of the aromatic hydrogens was obtained from the $^{1}H$-$^{13}C$ 2-D HETCOR experiments.

Table 7. $^{1}H$ NMR Assignments for Desulfonated/Acetylated Compound 34 (V)

| Delta, ppm | Integral | Couplings, Hz | Assignment |
|---|---|---|---|
| 1.35 | 3H | t, 6.9 | $-OCH_2CH_3$ |
| 2.00 | 3H | s | $ArNHC(O)CH_3$ |
| 4.05 | 2H | q, 6.9 | $-OCH_2CH_3$ |
| 6.78 | 2H | m | $H_{4'}$, $H_{5'}$ |
| 6.94 | 1H | m | $H_{3'}$ |
| 7.02 | 2H | m (AA') | $H_2$, $H_6$ |
| 7.02 | 1H | s (broad) | $(Ar)_2NH$ |
| 7.09 | 1H | m | $H_{6'}$ |
| 7.42 | 2H | m (XX') | $H_3$, $H_5$ |
| 9.77 | 1H | s | $ArNHC(O)CH_3$ |

s = singlet; t = triplet; q = quartet; m = multiplet

Data obtained from the $^{1}H$ decoupled $^{13}C$ NMR (Figure 10), the APT experiment, as well as the $^{1}H$-$^{13}C$ HETCOR experiments (Figures 12 and 13, directly coupled and long-range, respectively), are summarized in Table 8. Assignment of all $^{13}C$ resonances is possible from this data by applying the same logic used to assign the $^{13}C$ NMR data to Structures IVa and IVb, above. The long-range correlations to the amide proton are noteworthy as these allow assignment of the protonated carbons of the attached ring. The strongest correlation to the amide proton is with the $^{13}C$ resonance at delta 120.0 ppm, which is therefore assigned as $C_{3,5}$. Assignment of this resonance allows the chemical shift based assignment of the $^{1}H$ resonances for $H_{2,6}$ and $H_{3,5}$ to be checked. The direct $^{1}H$-$^{13}C$ correlation with the $^{13}C$ resonance at delta 120.0 ($C_{3,5}$) is with the $^{1}H$ resonance at delta 7.42, which therefore must be that of $H_{3,5}$ in agreement with the earlier assignment. Further proof was provided by irradiation of the amide proton resonance; an NOE to the $^{1}H$ resonance at delta 7.42 was observed, thus confirming the assignment of $H_{3,5}$ (Figure 14).

4.2.2 MS Characterization.

The EI and CI mass spectra (Figure 15) were obtained on the desulfonated/acetylated product from Compound 34 and were consistent with Structure V. The EI base peak is the molecular ion at m/z 270. The CI spectrum is predominantly the protonated molecular ion at m/z 271. Structural assignments for the CI fragmentation products are shown in Table 9.

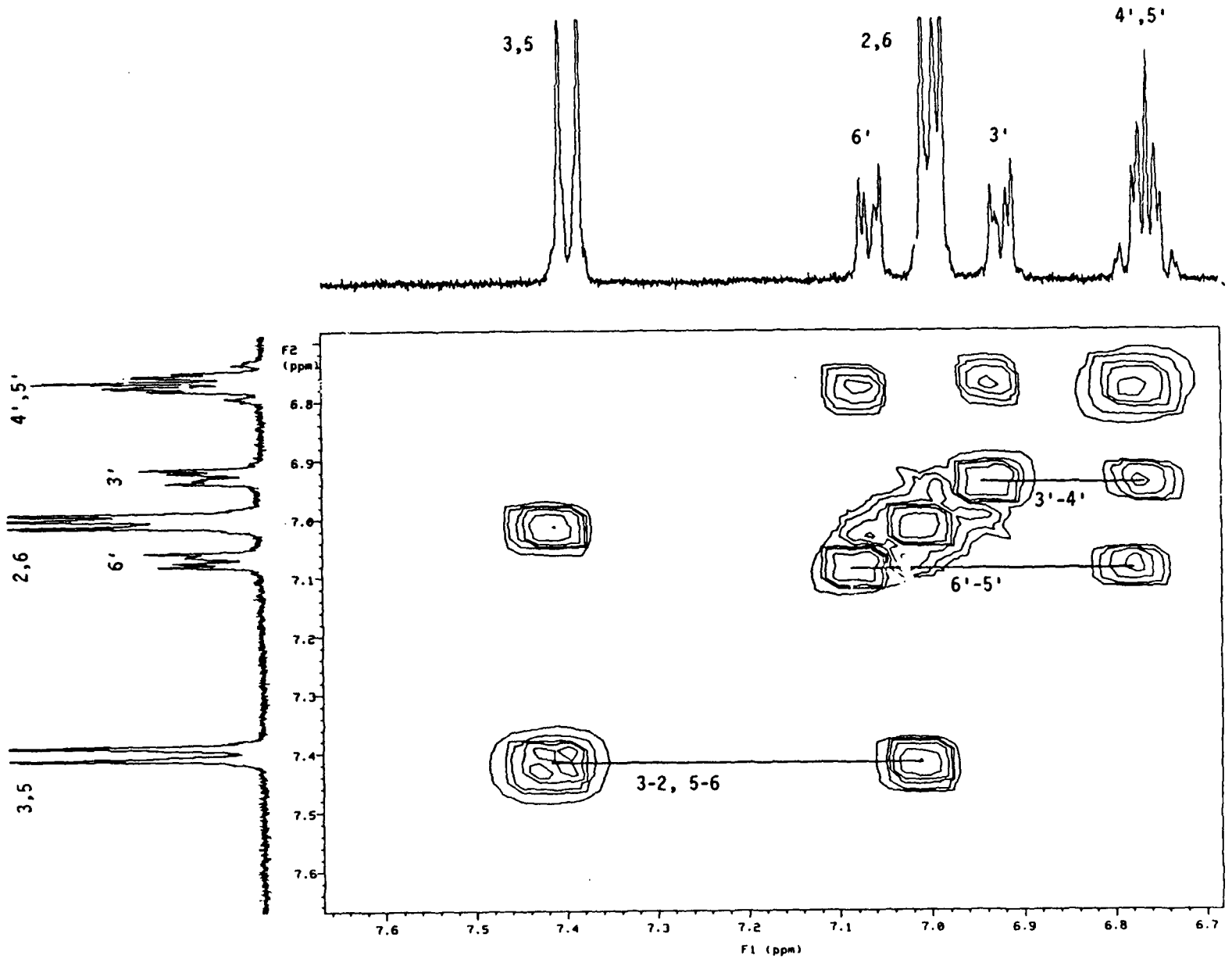



Figure 11. Desulfonated/Acetylated Compound 34 (V): $^{1}H$ COSY Spectrum, Aromatic Region

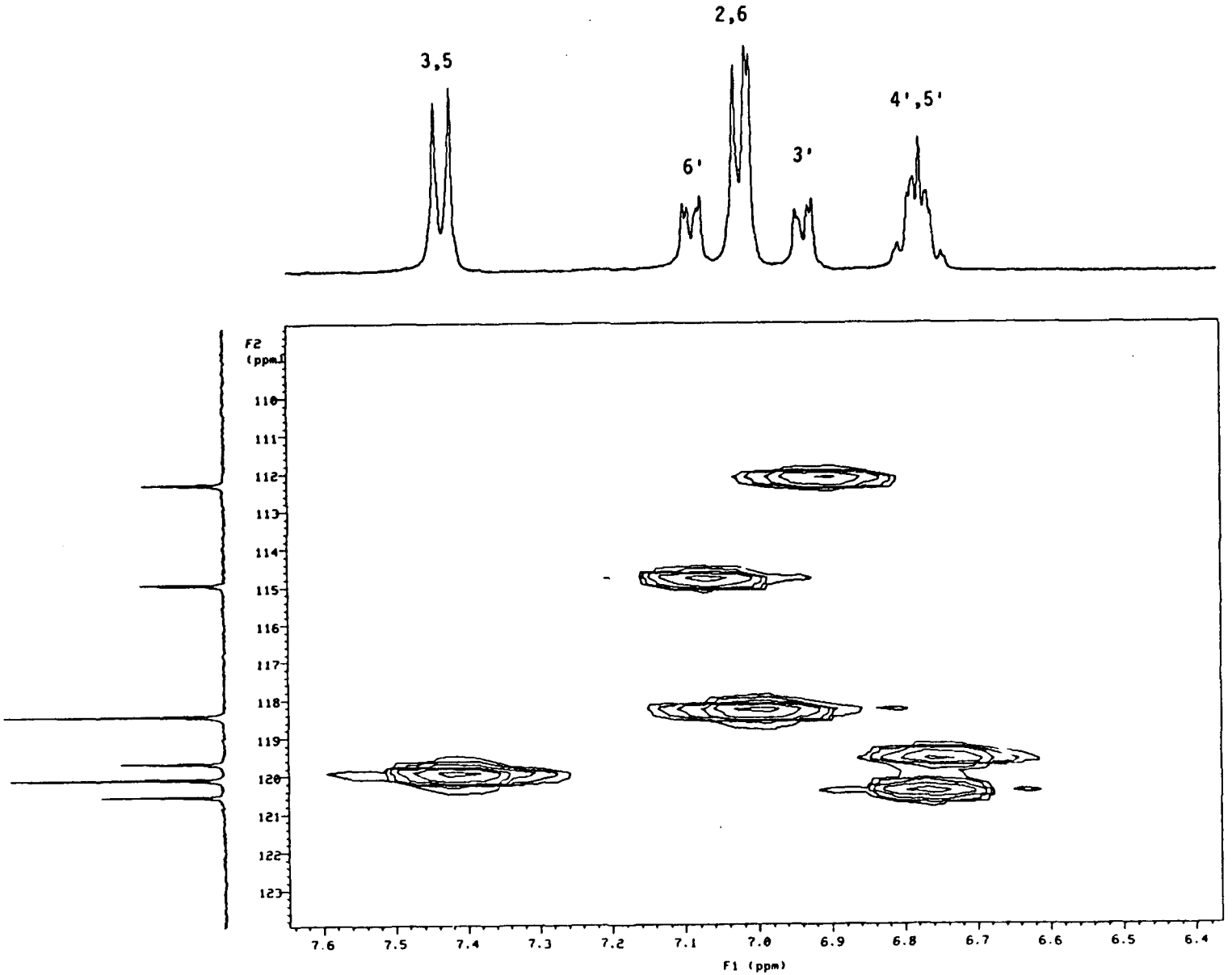



Figure 12. Desulfonated/Acetylated Compound 34 (V): Directly Coupled HETCOR Experiment, Aromatic Region

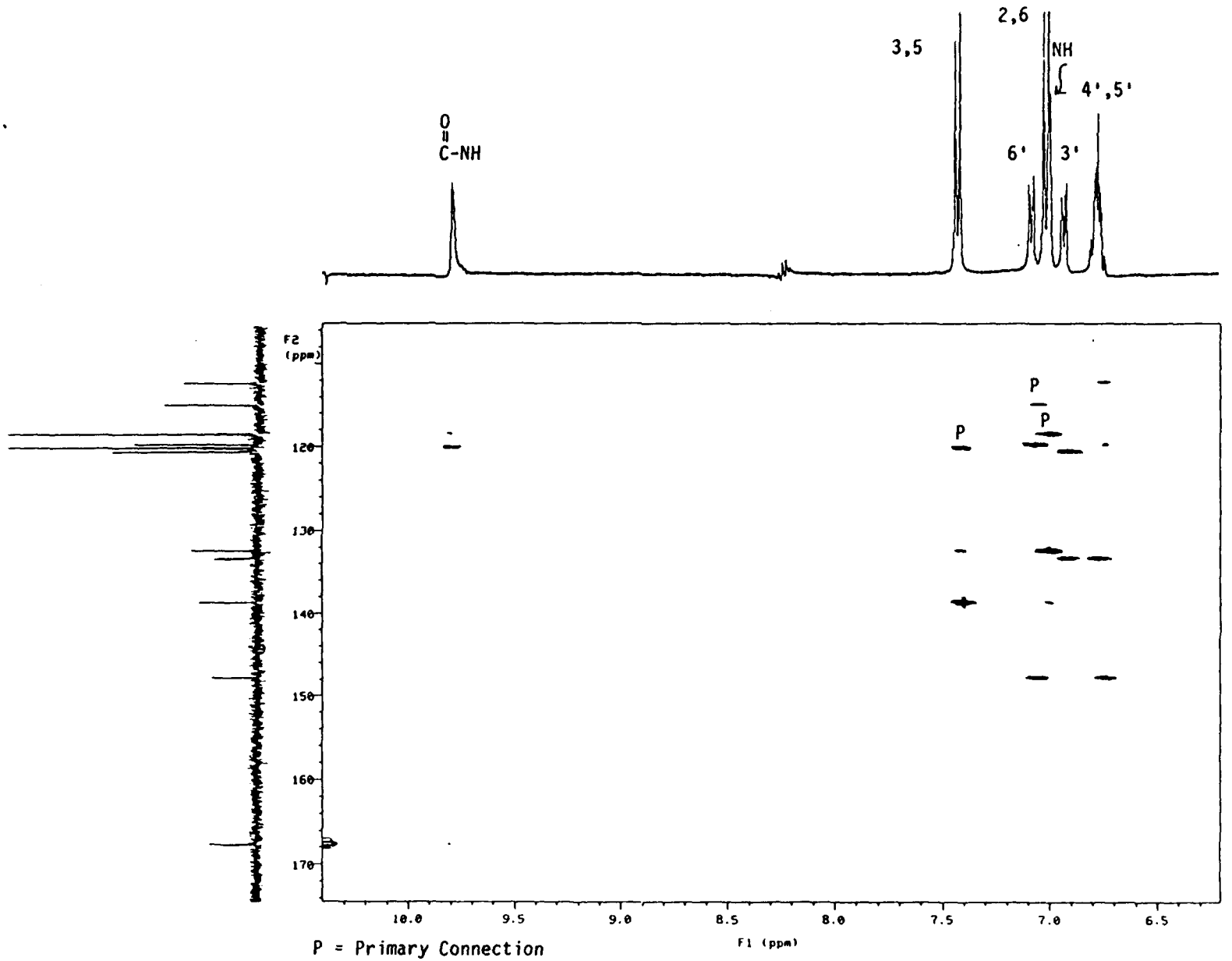



Figure 13. Desulfonated/Acetylated Compound 34 (V): Long-Range HETCOR Experiment, Aromatic Region

Table 8. $^{1}H$-$^{13}C$ Correlations for Desulfonated/Acetylated Compound 34 (V)

| Delta $^{13}C$, ppm | Directly Coupled Correlations | Long-Range Correlations | Assignment |
|---|---|---|---|
| 14.7 (q) | 1.35 ($-OCH_2\underline{CH_3}$) | 4.05 ($-OC\underline{H_2}CH_3$) | $-OCH_2\overline{CH_3}$ |
| 23.8 (q) | 2.00 ($-C(O)\overline{CH_3}$) | - | $-C(O)\overline{CH_3}$ |
| 63.6 (t) | 4.05 ($-OCH_2\overline{CH_3}$) | 1.35 ($-OCH_2\underline{CH_3}$) | $-O\overline{CH_2}CH_3$ |
| 112.2 (d) | 6.94 ($H_3'$) | 6.78 (w, $H_4'$ or $H_5'$) | $\overline{C_3}'$ |
| 114.9 (d) | 7.09 ($H_6'$) | - | $C_6'$ |
| 118.4 (d, 2C) | 7.02 ($H_{2,6}$) | 7.02 (s, $H_{6,2}$)<br>9.8 (w, amide) | $C_{2,6}$ |
| 119.6 (d) | 6.78 ($H_4'$ or $H_5'$) | 7.09 (s, $H_6'$) | $C_4'$ |
| 120.0 (d, 2C) | 7.42 ($H_{3,5}$) | 7.42 (s, $H_{5,3}$)<br>9.8 (s, amide) | $C_{3,5}$ |
| 120.5 (d) | 6.78 ($H_4'$ or $H_5'$) | 6.94 (s, $H_3'$) | $C_5'$ |
| 132.3 (quat) | - | 7.02 (s, $H_{2,6}$)<br>7.42 (w, $H_{3,5}$) | $C_4$ |
| 133.2 (quat) | - | 6.78 (s, $H_4'$ or $H_5'$)<br>6.94 (s, $H_3'$) | $C_1'$ |
| 138.5 (quat) | - | 7.42 (s, $H_{3,5}$)<br>7.02 (w, $H_{2,6}$) | $C_1$ |
| 147.7 (quat) | - | 6.78 (s, $H_4'$ or $H_5'$)<br>7.09 (s, $H_6'$) | $C_2'$ |
| 167.5 (quat) | - | 9.8 (w, amide) | C=O |

d = doublet; t = triplet; q = quartet; quat = quaternary carbon;
w = weak; s = strong

## 4.3 Acetylated Compound 34 (VI).

### 4.3.1 NMR Characterization.

The $^{1}H$, $^{1}H$ COSY, $^{13}C$, HETCOR, and long-range HETCOR spectra of VI are shown in Figures 16-20b, respectively. Two features of the $^{1}H$ spectrum warrant comment. First, a smaller resonance (ca. 20%) with the same multiplicity is found for each major peak. A similar duplication of peaks is also apparent in the $^{13}C$ spectrum. The most reasonable interpretation of this feature is that slow exchange between the syn- and anti- regio-isomers of the secondary amine acetamide (shown below) results in separate spectra for each isomer.



Text continues on page 50

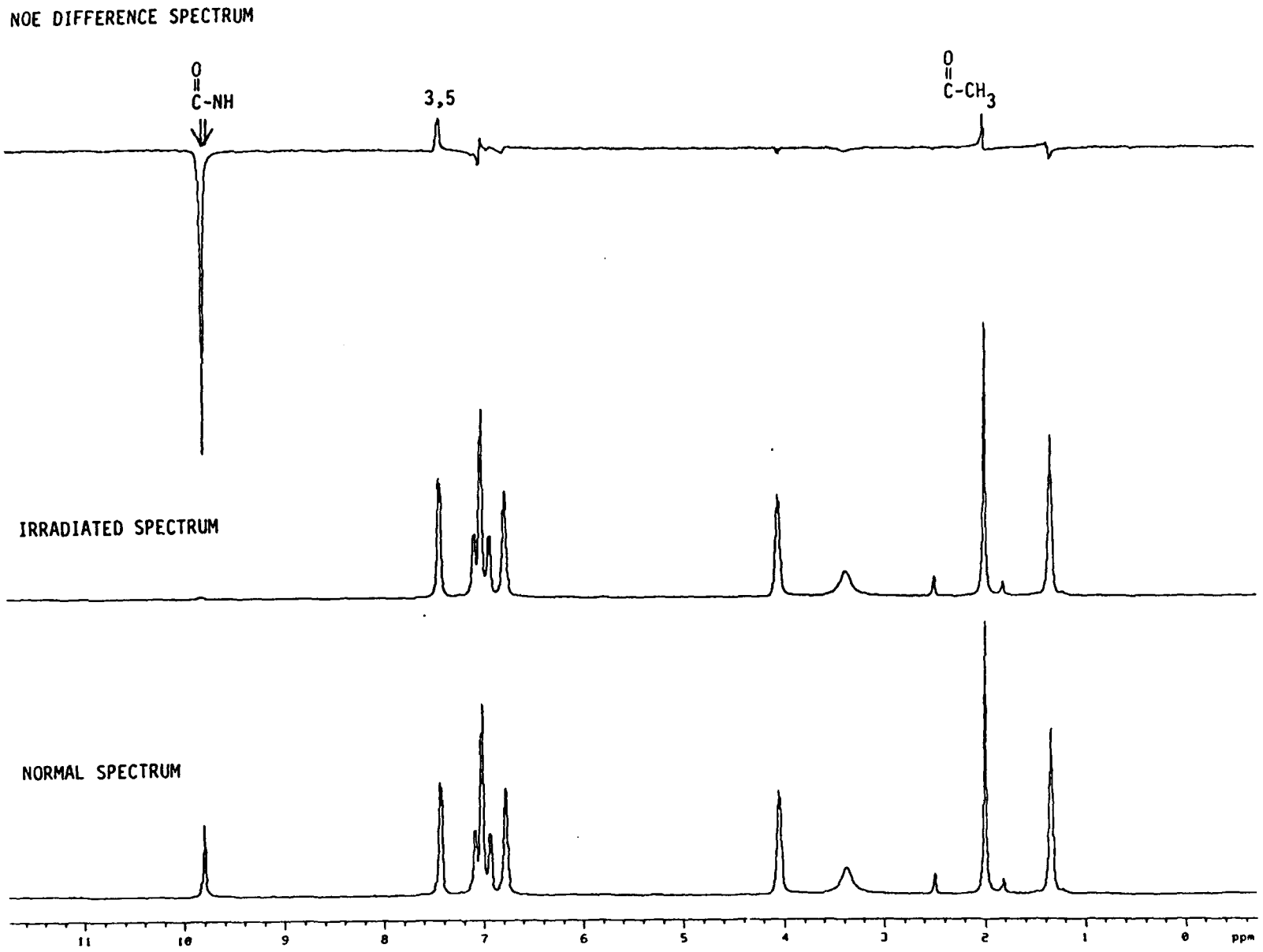



Figure 14. Desulfonated/Acetylated Compound 34 (V): 1-D NOE Experiment, Irradiation of Amide Proton

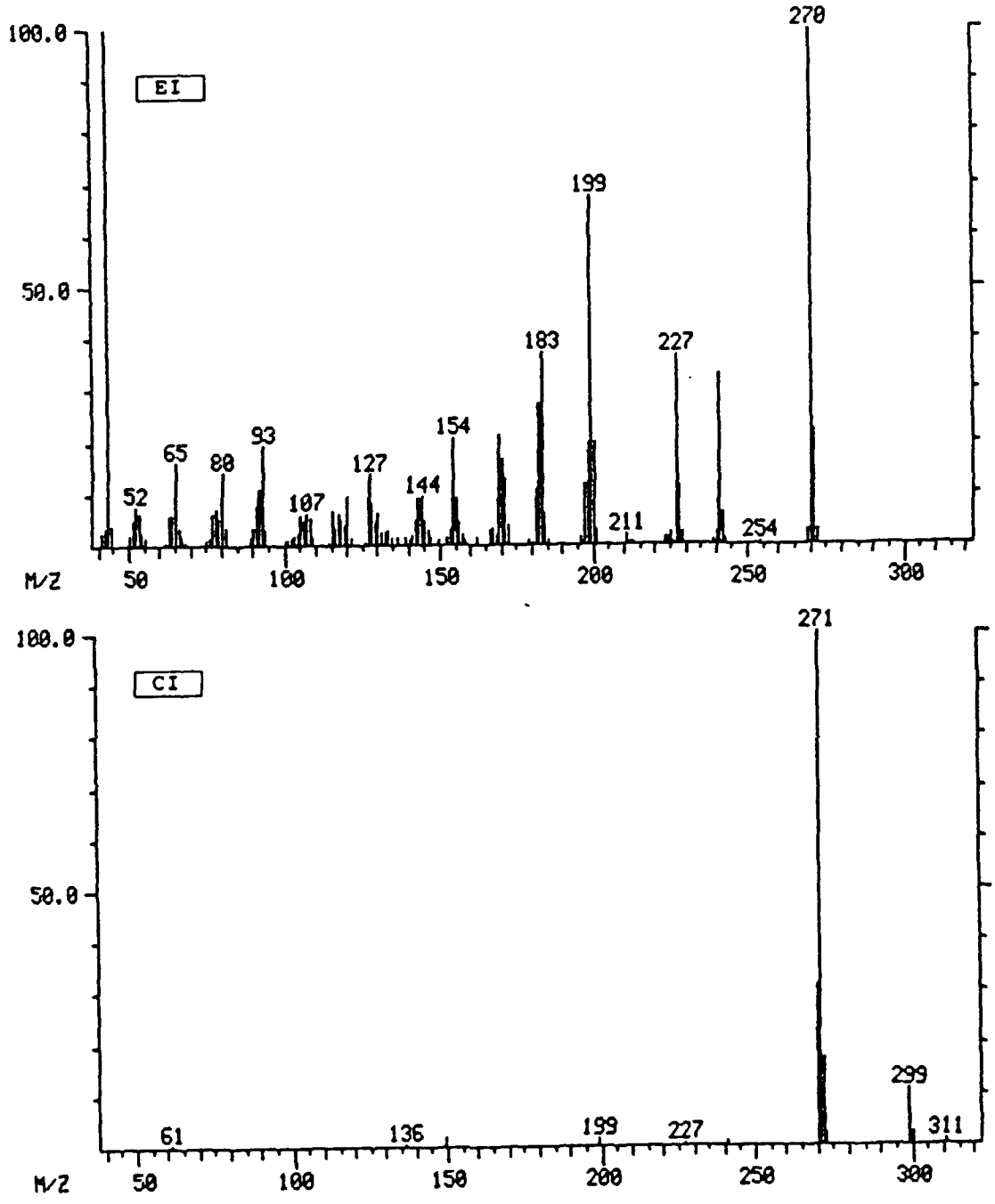
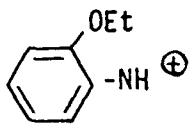
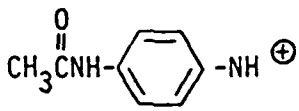
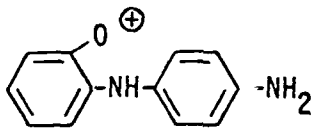
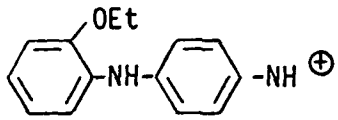
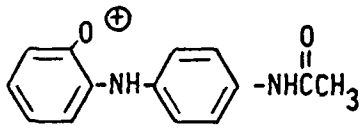
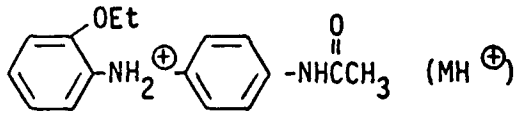



Figure 15. Desulfonated/Acetylated Compound 34 (V): EI and CI Mass Spectra

Table 9. Desulfonated/Acetylated Compound 34 (V) CI Mass Spectral Fragmentation Ions

| M/Z | Structural Assignment | Relative Intensity |
|---|---|---|
| 136 |  | 1 |
| 149 |  | 2 |
| 199 |  | 2 |
| 227 |  | 1 |
| 241 |  | 1 |
| 271 |  | 100 |
| 299 | $M + C_2H_5^{\oplus}$ | 11 |
| 311 | $M + C_3H_5^{\oplus}$ | 1 |

M = Molecular Ion, 270.

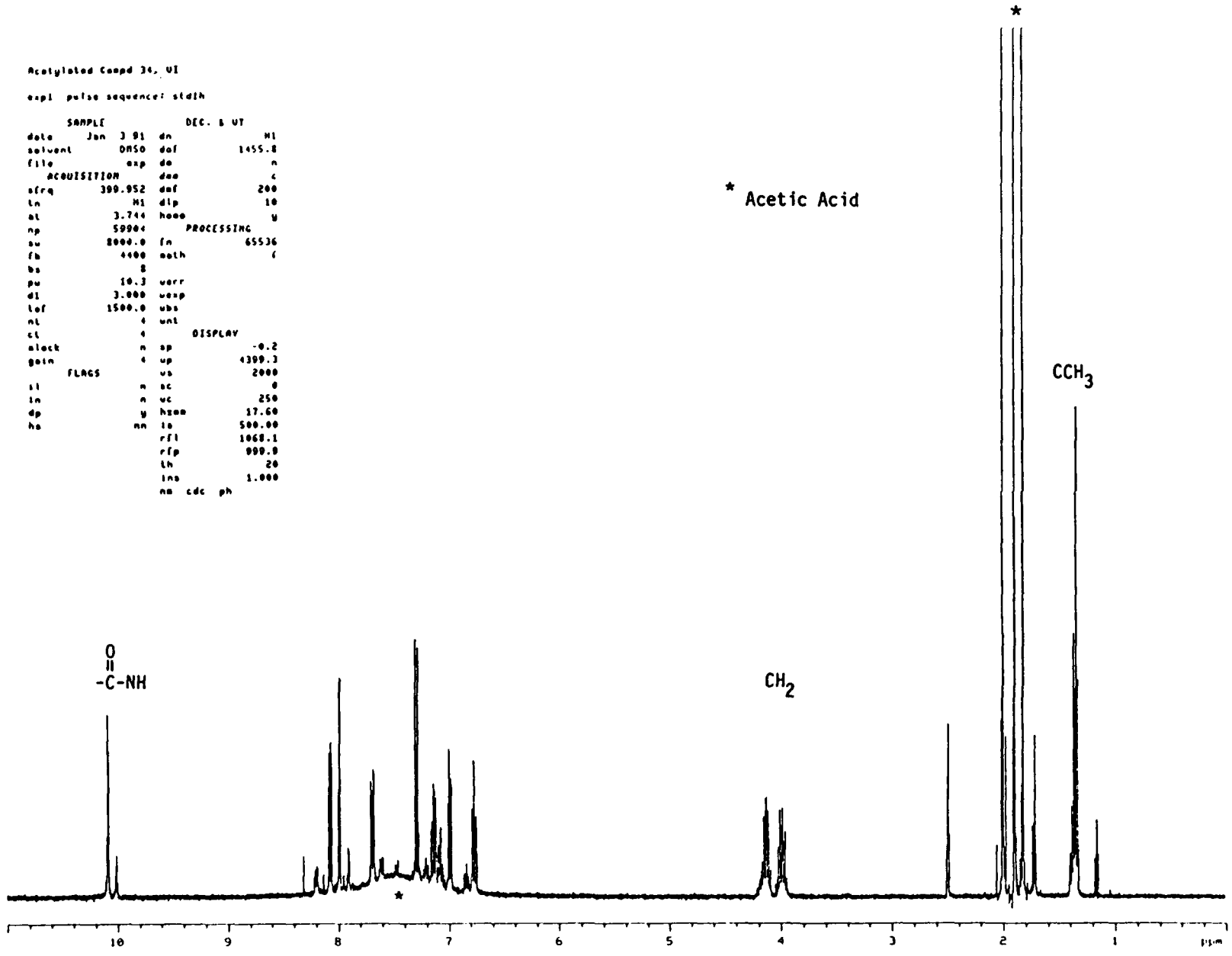



Figure 16a. Diacetylated Compound 34 (VI): $^{1}H$ NMR Spectrum in DMSO-$d_6$

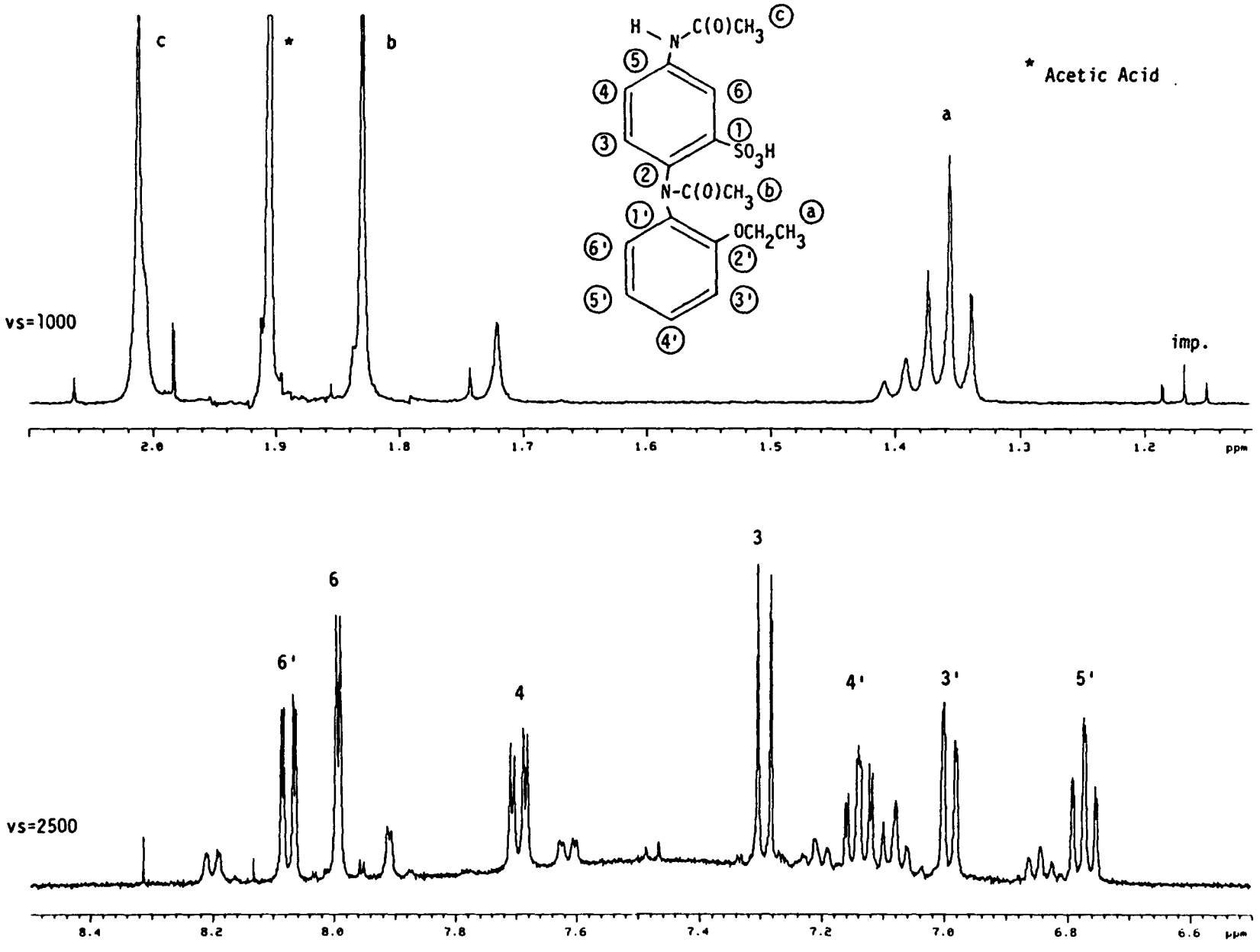



Figure 16b. Diacetylated Compound 34 (VI): Expanded $^{1}H$ NMR Spectrum

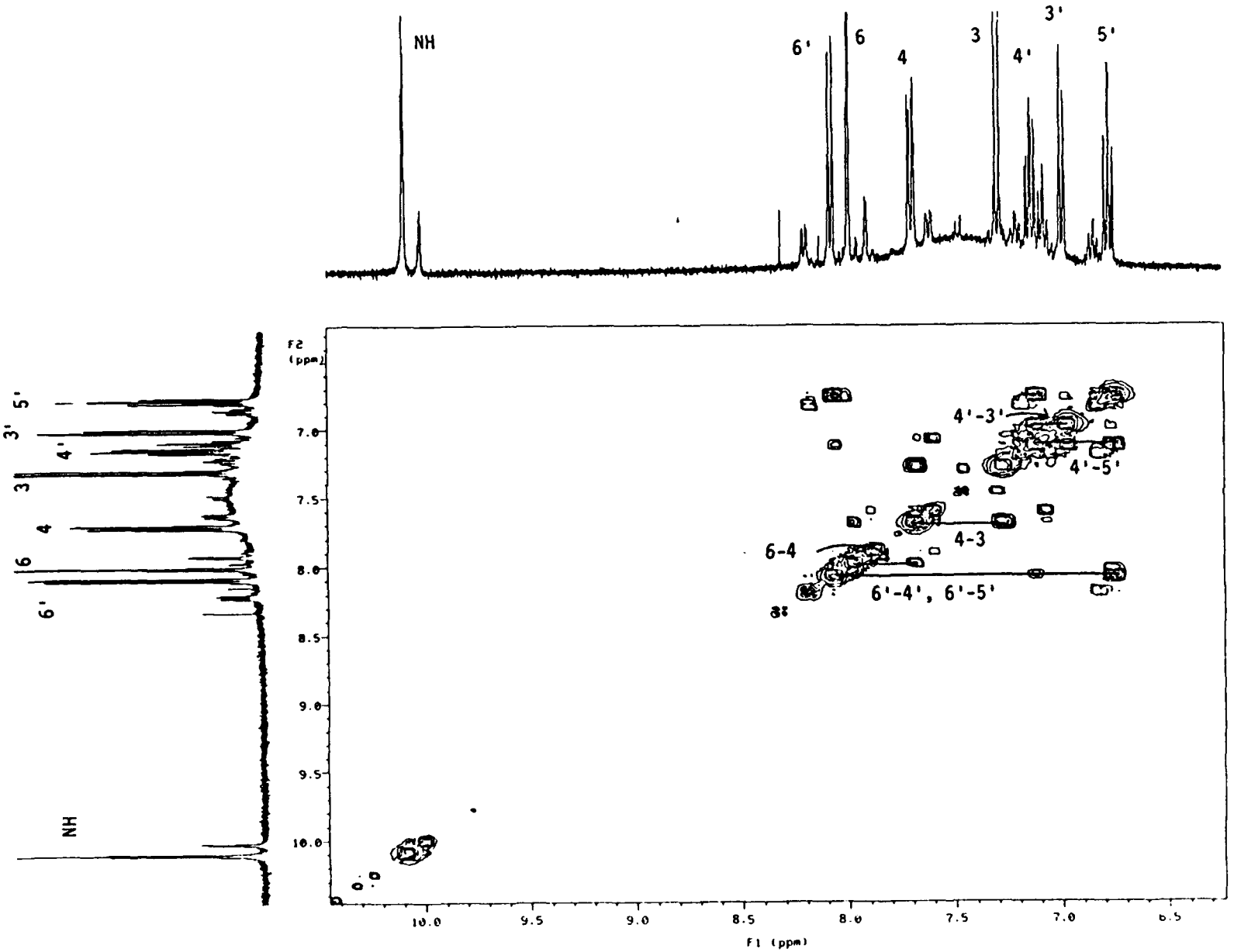



Figure 17. Diacetylated Compound 34 (VI): $^{1}H$ COSY Spectrum, Aromatic and Amide Regions

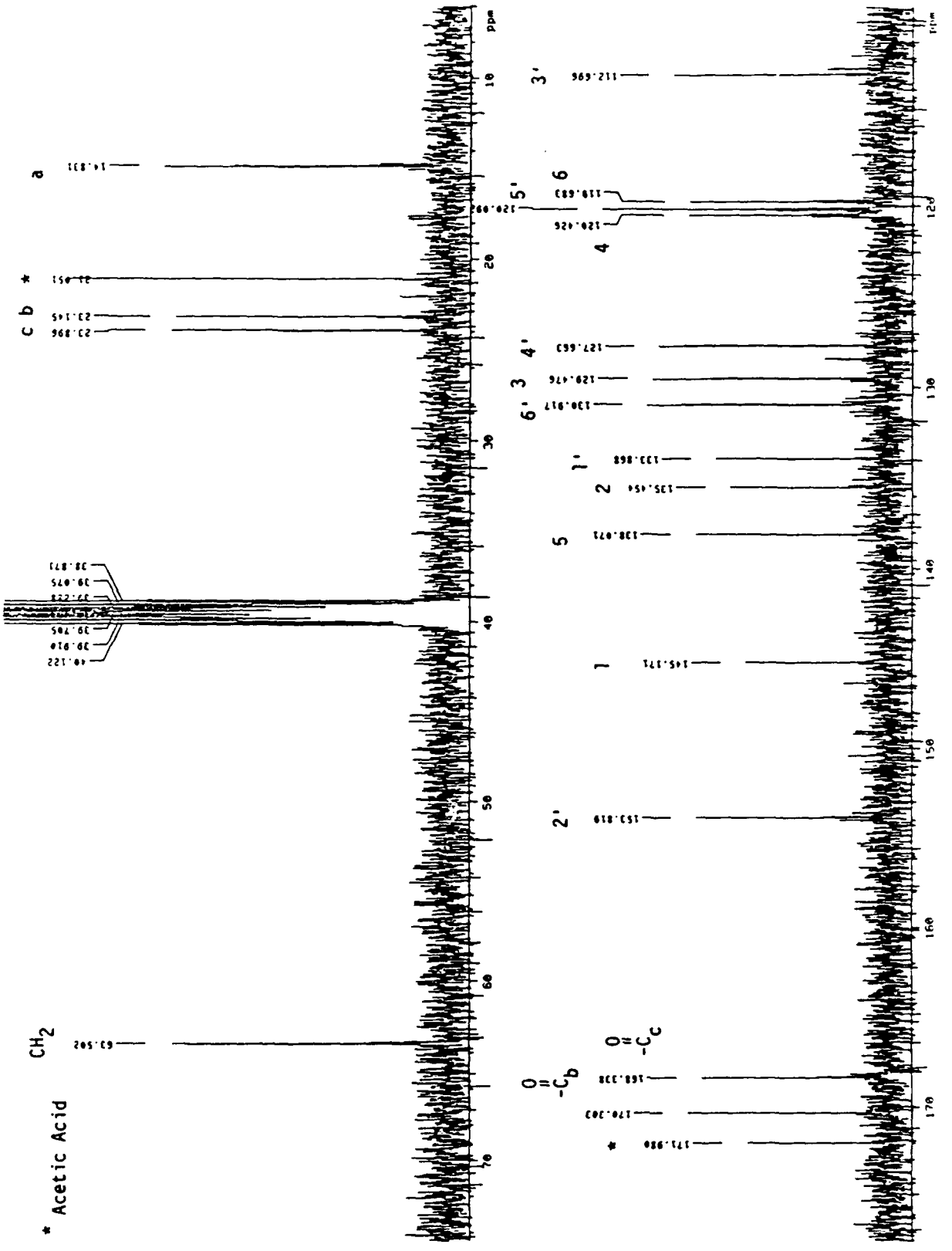



Figure 18. Diacetylated Compound 34 (VI): $^{13}C$ NMR Spectrum in DMSO-$d_6$

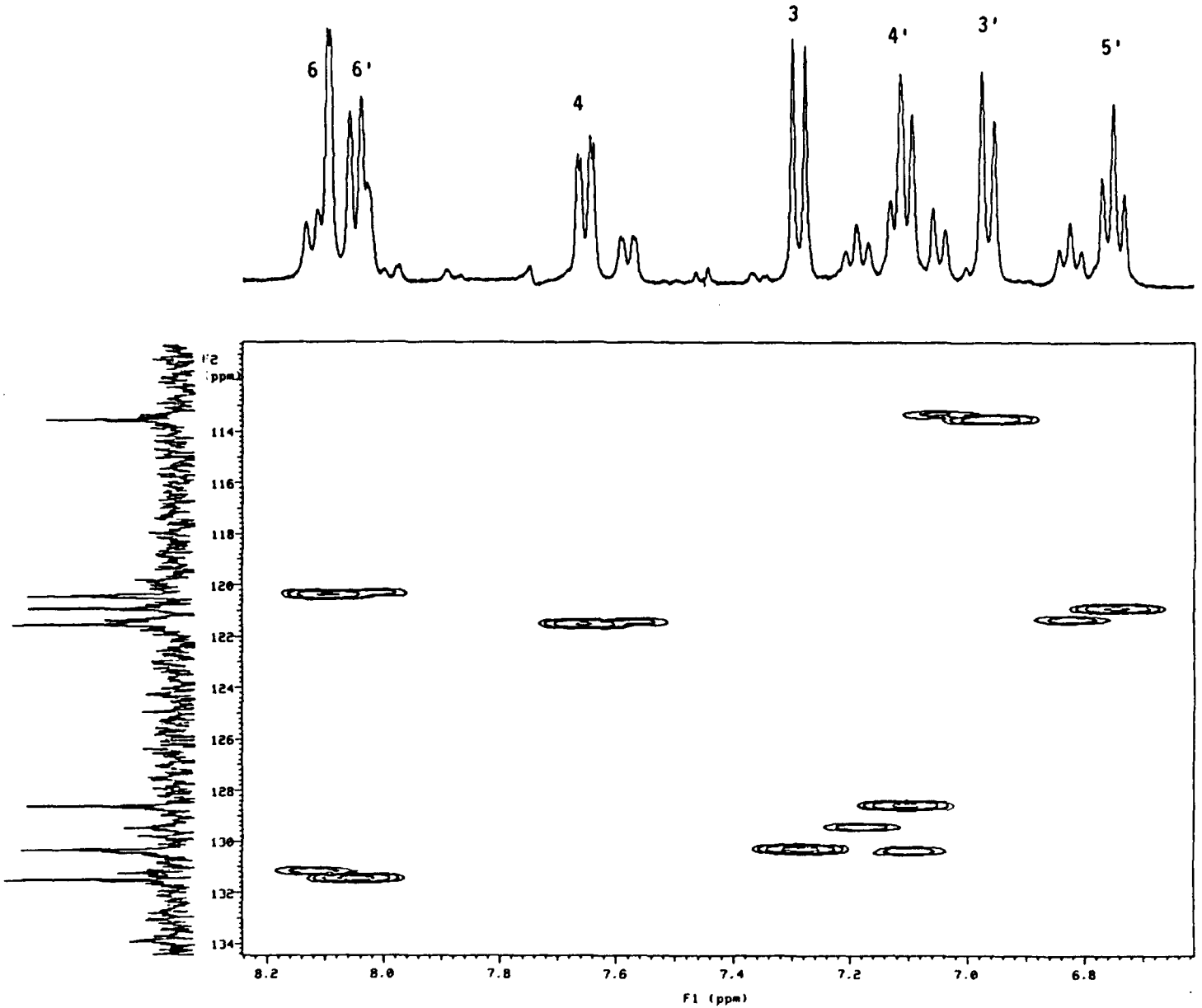



Figure 19. Diacetylated Compound 34 (VI): Directly Coupled HETCOR Experiment, Aromatic Region

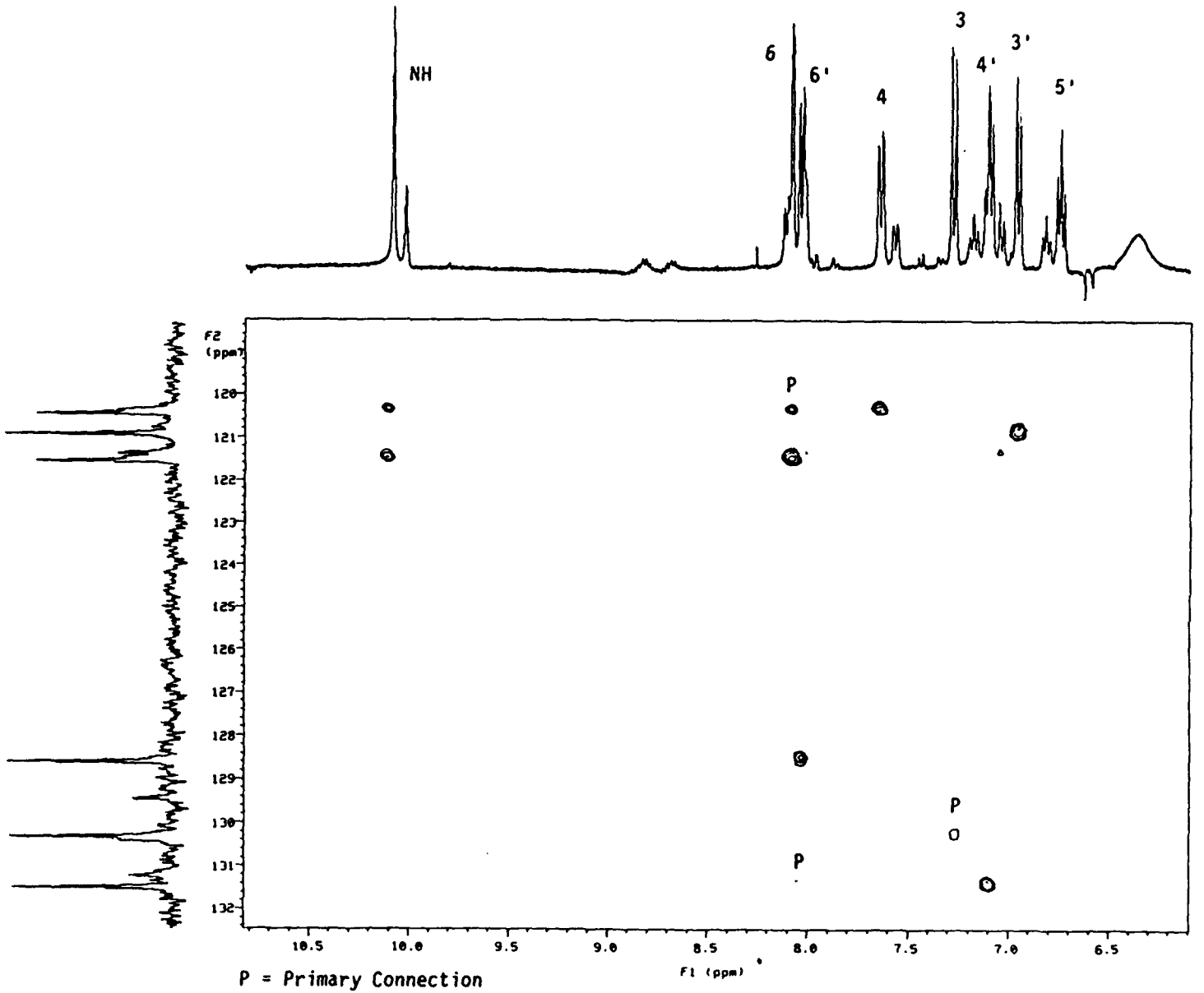



Figure 20a. Diacetylated Compound 34 (VI): Long-Range HETCOR Experiment, Aromatic Region

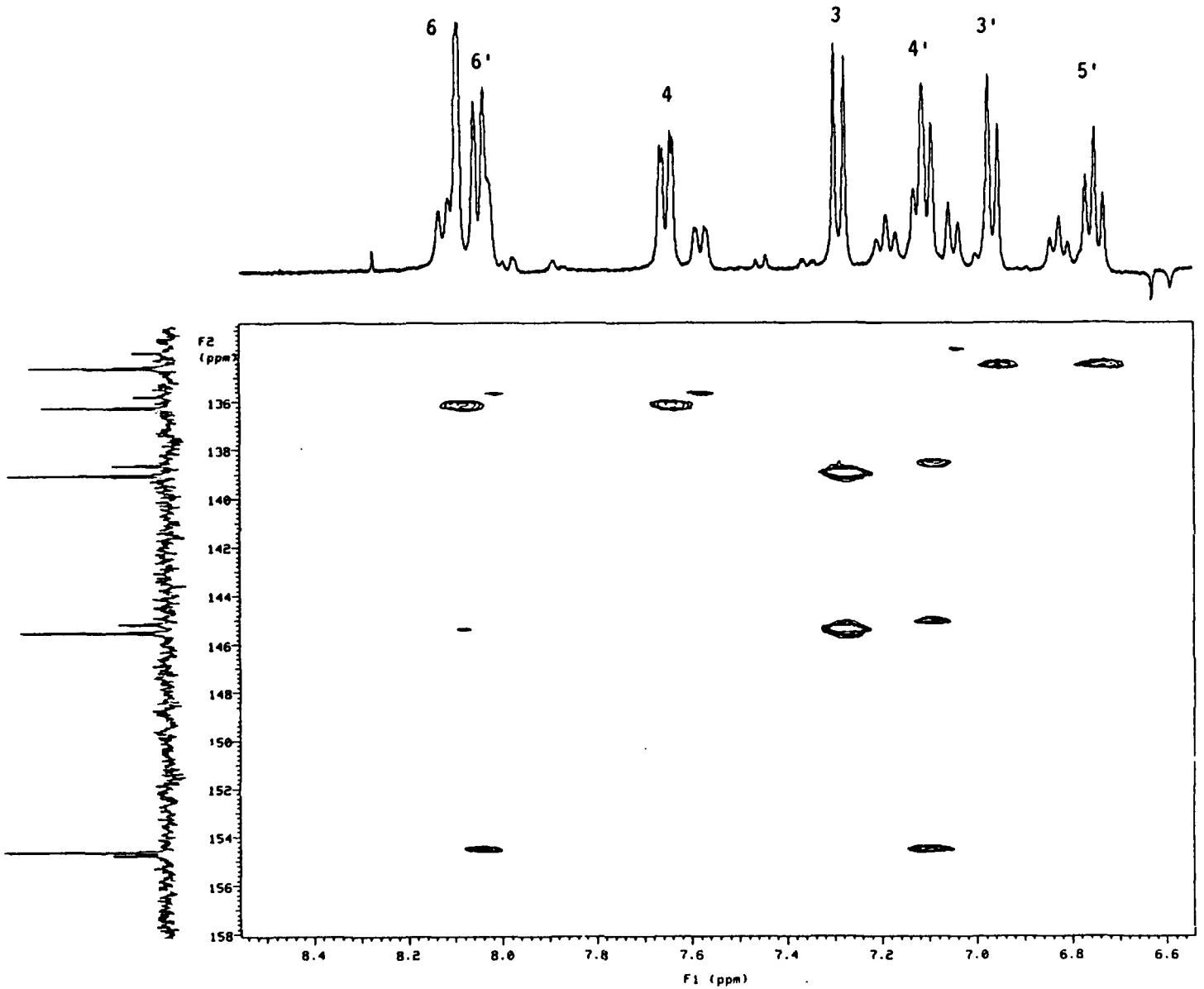
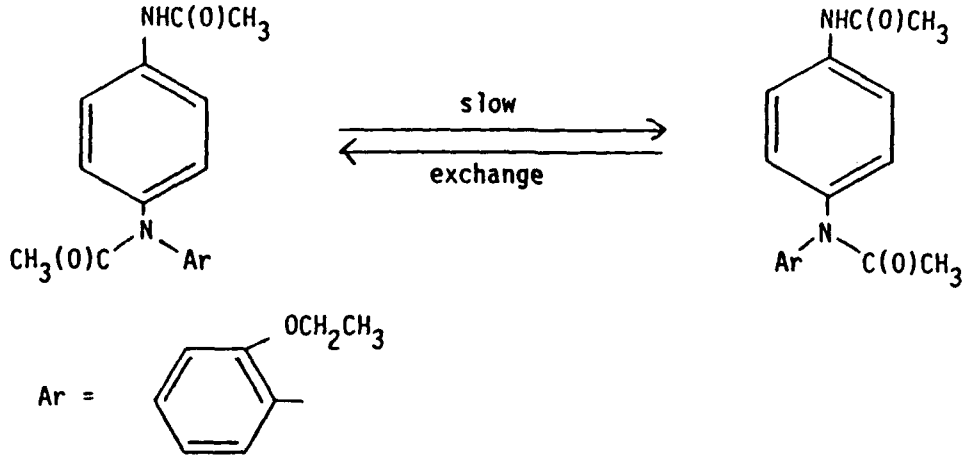



Figure 20b. Diacetylated Compound 34 (VI): Long-Range HETCOR Experiment, Aromatic Region




Heating the sample to 80 °C causes coalescence of each pair of peaks, confirming that the resonances in each pair are linked by exchange. Second, as indicated in both the $^{1}H$ and $^{13}C$ spectra, three acetate groups appear to be in the sample. In all other respects, the spectra are consistent with the N,N'-diacetamide of IVa or IVb. The extra acetate group arises from the presence of one equivalent of acetic acid that is not removed by freeze-drying. An increase in the intensity of the resonance at delta 1.90 ppm in the $^{1}H$ spectrum and those at deltas 21.1 and 172.0 in the $^{13}C$ spectrum after adding acetic acid to the sample allowed assignment of these resonances.

(Note: The following discussion is limited to the major regio-isomer of VI.)

Assignment of the $^{1}H$ NMR spectrum is straightforward. Spin systems can be identified in the $^{1}H$ COSY spectrum, and assignment of individual resonances (Table 10) is possible by comparison with the spectra for Compound 34 and V. The resonances at delta 2.00 and 1.82 ppm are assigned as the secondary and tertiary acetamide methyls, respectively. The chemical shift of the secondary acetamide methyl in V (delta 2.00) provides the basis for this assignment. The HETCOR and long-range HETCOR experiments allow assignment of the $^{13}C$ spectrum (Table 11). The only ambiguous assignments are those for the resonances at deltas 138.1 and 145.2 for $C_5$ and $C_1$, respectively. The final assignment of these peaks was made using substituent effects (see below) after the position of the sulfonic acid moiety was determined.

Differentiation between the two possible structures for VI (i.e., VIa and VIb) is possible by establishing a through-space connection of the amide proton with protons on the aromatic ring. Irradiation of the amide $^{1}H$ resonance (delta 10.08) provided enhancement of the aromatic proton resonances at deltas 7.98 ($H_6$) and 7.69 ($H_4$) (Figure 21). Clearly, both $H_6$ and $H_4$ are ortho to the $NHC(O)CH_3$ moiety, and the correct structure for diacetylated Compound 34 is VIb. A NOESY experiment, the 2-D version of the NOE experiment, was also performed. The NOE correlations between the amide proton resonance and those of $H_4$ and $H_6$ are also observed in this experiment (boxed areas, Figure 22). In addition to NOE correlations, NOESY spectra exhibit cross peaks between resonances linked by chemical exchange. Therefore, strong correlations are present between each of the resonances of the major regio-isomer of VI and its

Table 10. $^{1}H$ NMR Assignments for the Major Regio-Isomer of Diacetylated Compound 34 (VI)

| Delta $^{1}H$, ppm | Integral | Couplings, Hz | Assignment |
|---|---|---|---|
| 1.35 | 3H | t, 7.0 | $-OCH_2CH_3$ |
| 1.82 | 3H | s | $(Ar)_2N-C(O)CH_3$ |
| 1.90 | 3H | s | $CH_3C(O)OH$ |
| 2.00 | 3H | s | $ArNHC(O)CH_3$ |
| 3.99 | 1H | m | $-OCH_aH_bCH_3$ |
| 4.13 | 1H | m | $-OCH_aH_bCH_3$ |
| 6.76 | 1H | t, 7.6; d, 1.2 | $H_5'$ |
| 6.98 | 1H | d, 8.0; d, 1.2 | $H_3'$ |
| 7.13 | 1H | d, ~8; d, ~8; d, 1.6 | $H_4'$ |
| 7.28 | 1H | d, 8.6 | $H_3$ |
| 7.69 | 1H | d, 8.6; d, 2.6 | $H_4$ |
| 7.98 | 1H | d, 2.6 | $H_6$ |
| 8.07 | 1H | d, 8.0., d, 1.6 | $H_6'$ |
| 10.08 | 1H | s | $ArNHC(O)CH_3$ |

s = singlet; d = doublet; t = triplet; m = multiplet

Table 11. $^{1}H$-$^{13}C$ Correlations for the Aromatic Resonances of the Major Regio-Isomer of Diacetylated Compound 34 (VI)*

| Delta $^{13}C$, ppm | Directly Coupled Correlations | Long-Range Correlations | Assignment |
|---|---|---|---|
| 112.7 (d) | 6.97 ($H_3'$) | | $C_3'$ |
| 119.7 (d) | 8.09 ($H_6$) | 7.65 (s, $H_4$) | $C_6$ |
| | | 10.1 (s, $ArNHCOCH_3$) | |
| 120.1 (d) | 6.75 ($H_5'$) | 6.97 (s, $H_3'$) | $C_5'$ |
| 120.4 (d) | 7.65 ($H_4$) | 8.09 (s, $H_6$) | $C_4$ |
| | | 10.1 (s, $ArNHCOCH_3$) | |
| 127.7 (d) | 7.10 ($H_4'$) | 8.04 (s, $H_6'$) | $C_4'$ |
| 129.5 (d) | 7.28 ($H_3$) | | $C_3$ |
| 130.9 (d) | 8.04 ($H_6'$) | 7.10 (s, $H_4'$) | $C_6'$ |
| 133.9 (quat) | - | 6.97 (s, $H_3'$) | $C_1'$ |
| | | 6.75 (s, $H_5'$) | |
| 135.5 (quat) | - | 8.09 (s, $H_6$) | $C_2$ |
| | | 7.65 (s, $H_4$) | |
| 138.1 (quat) | - | 7.28 (s, $H_3$) | $C_5$ |
| 145.2 (quat) | - | 7.28 (s, $H_3$) | $C_1$ |
| | | 8.09 (w, $H_6$) | |
| 153.8 (quat) | - | 8.04 (s, $H_6'$) | $C_2'$ |
| | | 7.10 (s, $H_4'$) | |

d = doublet; quat = quaternary carbon; s = strong; w = weak

*These HETCOR experiments were obtained using a more concentrated solution than the 1-D ($^{1}H$ and $^{13}C$) spectra. Some variation in chemical shifts with concentration are apparent. In particular, the doublet (J=2.6 Hz) for $H_6$ moves downfield to 8.09 ppm. However, there is no ambiguity in assignments. Reported $^{13}C$ shifts are for the least concentrated (15 mg/mL) solution.

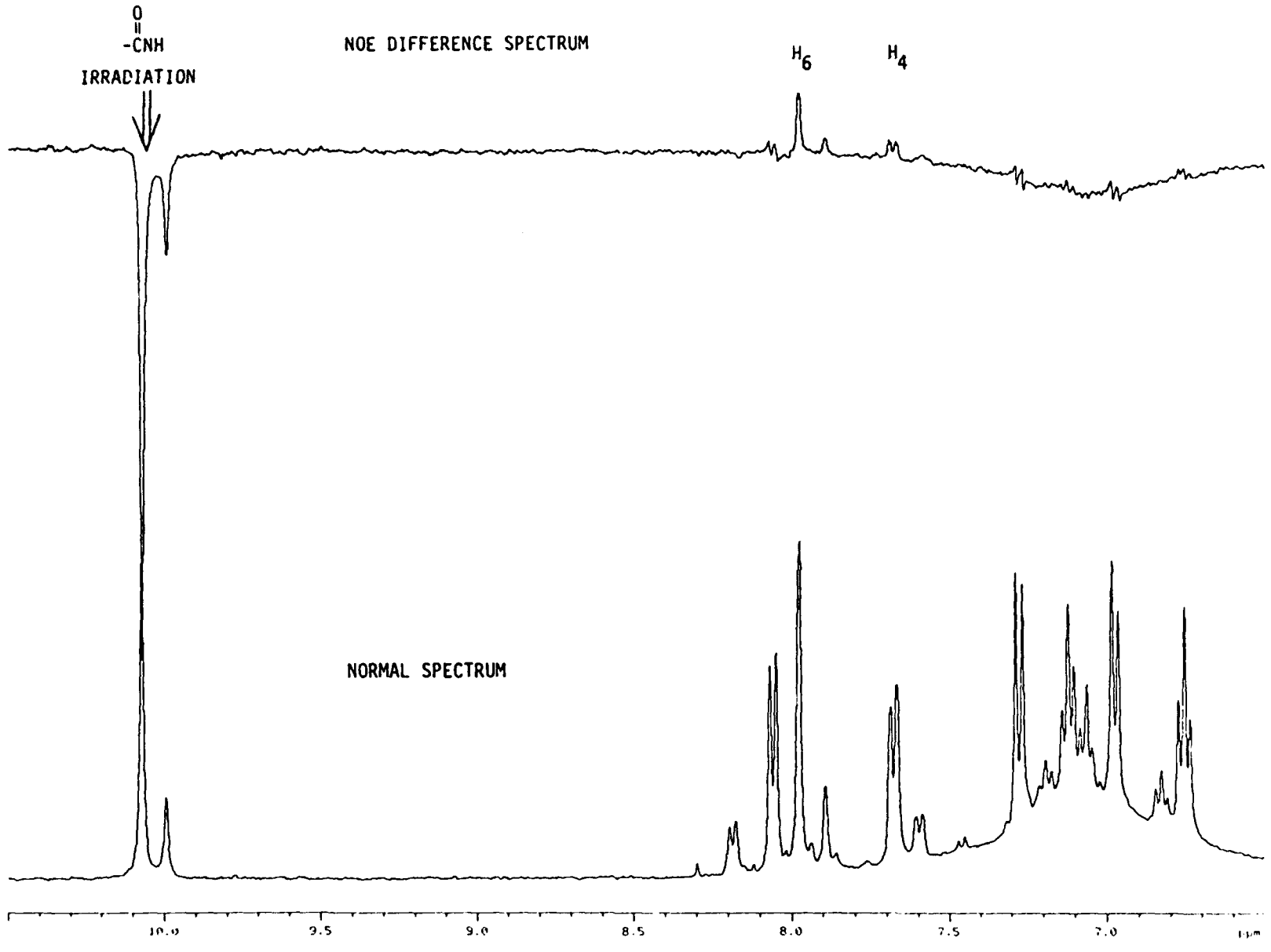



Figure 21. Diacetylated Compound 34 (VI): 1-D NOE Experiment, Irradiation of Amide Proton

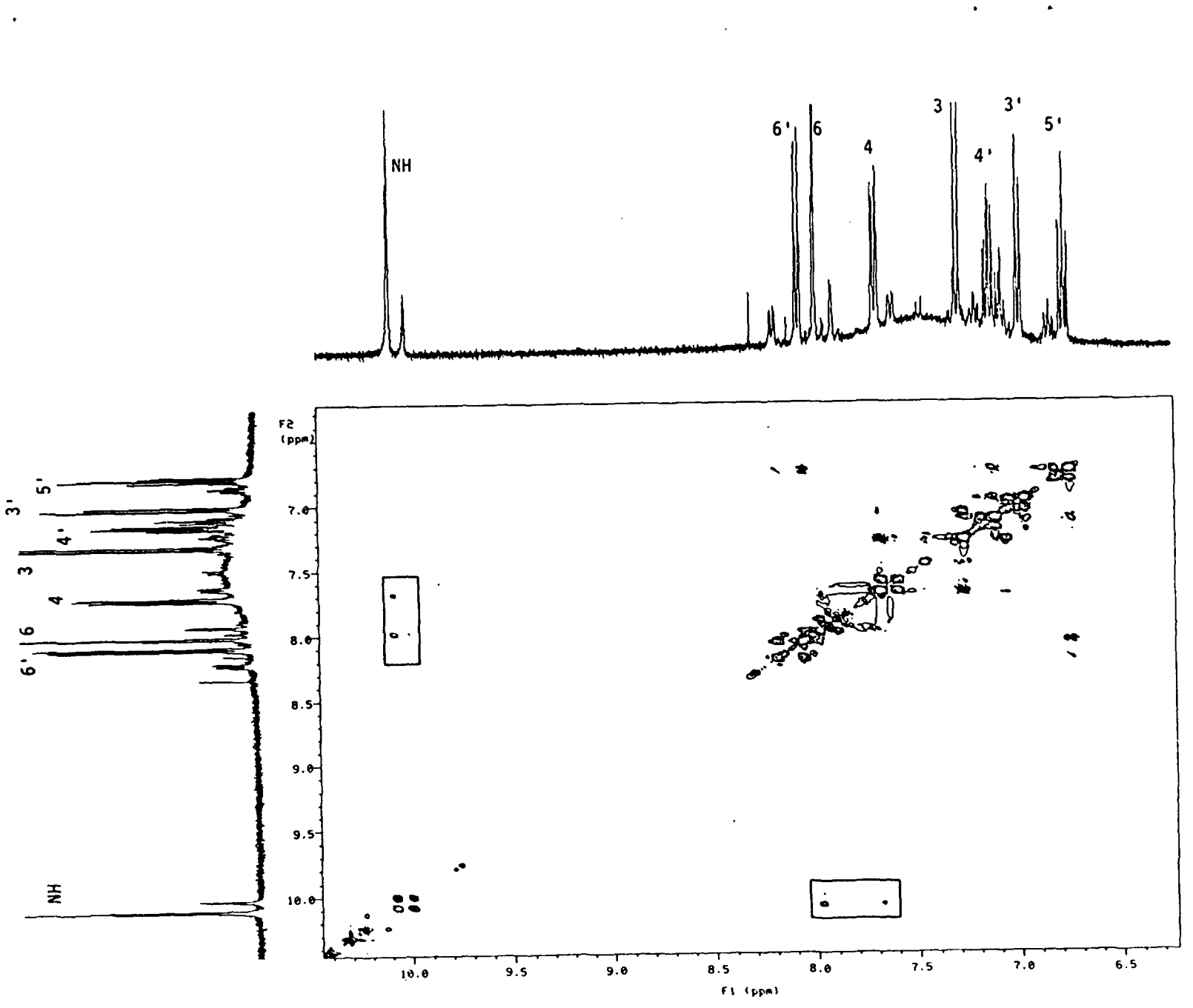
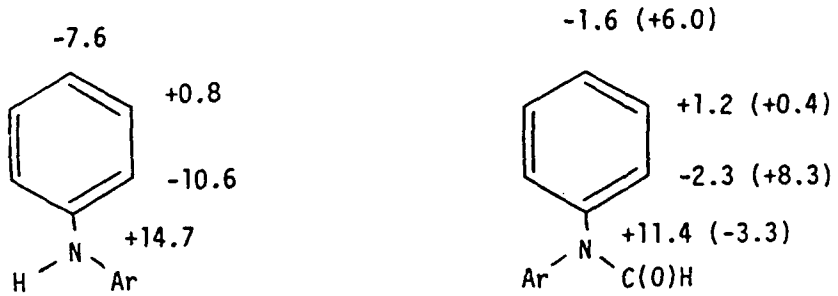



Figure 22. Diacetylated Compound 34 (VI): NOESY Spectrum. Boxed-in areas denote through-space correlations with the amide proton.

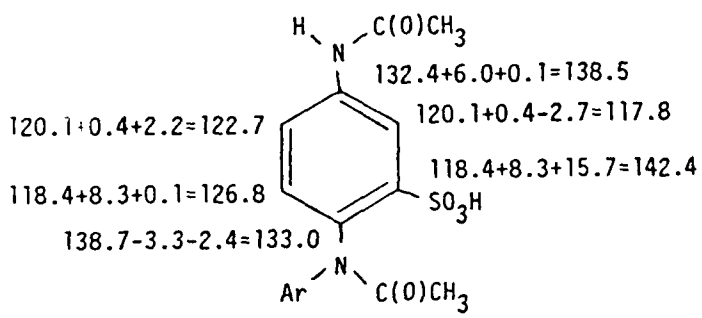
smaller counterpart resonance for the minor regio-isomer. This experiment complements the elevated temperature spectrum, and both demonstrate exchange between the major and minor species (regio-isomers) in solution.

Determining the arrangement of substituents in VI was also possible by analysis of the long-range $^{1}H$-$^{13}C$ correlations involving the amide proton. The 2-D long-range HETCOR spectrum (Figure 20) exhibits correlations between this proton and two protonatad carbon resonances ($C_4$ and $C_6$). Thus, this acetamide substitutent has two ortho-hydrogens, further confirming VIb as the product obtained from diacetylation of Compound 34. Therefore, the correct structure for Compound 34 is 5-amino-2(2'-ethoxyphenyl)aminobenzenesulfonic acid or 4-amino-2'-ethoxy-2-sulfo diphenylamine, IVb.

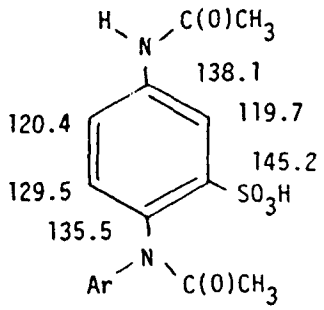
Estimates of the chemical shifts for $C_1$ and $C_5$ can be obtained by substituent effect calculations using V as the model compound. The effects of acetylation of the secondary amine can be estimated from the difference in the substituent effects of phenylamino and N-formyl phenylamino groups[13] and are shown below. The numbers in parentheses represent the differences between the amine and the formamide substituent values.




The chemical shifts for the trisubstituted ring of VIb, calculated by applying substituent effects for amide formation and those for sulfonation[13] to the shifts of V, are shown below. On the basis of these calculations, the resonances at deltas 138.1 and 145.2 are assigned to $C_5$ and $C_1$, respectively.




Calculated




Observed

The good agreement between all calculated and experimental shifts for VIb contrasts with the situation for IVb above. In particular, the difference between the two values for the sulfonic acid substituted carbon (ca. 17 ppm!) deserves comment. The presence of both sulfonic acid and amine groups in

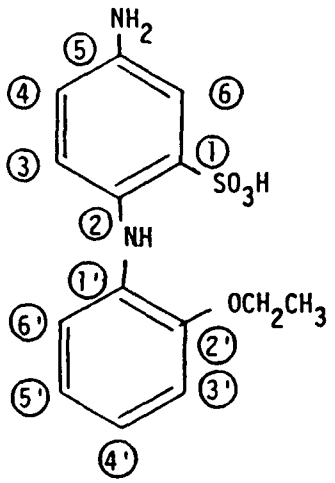
Compound 34 suggests that the compound would exist as a zwitterion rather than as the undissociated acid. In this case, the substituent effects used for IVb would not be appropriate for calculation of the $^{13}C$ shifts of Compound 34.

4.3.2 MS Characterization.

The EI and CI mass spectra were obtained for the diacetylated derivative of Compound 34 and are shown in Figure 23. The spectra indicate that this compound is not stable under MS conditions. Two early peaks were noted in the DIP profile, which correspond to the spectra of acetic acid, suggesting that one or both acetyl groups may be lost during heating. The spectra of the main DIP peak shown in Figure 23 show EI ions at m/z 228 and 270 corresponding to desulfonated Compound 34 and desulfonated monoacetylated Compound 34, respectively. A small ion occurs at m/z 312, which would correspond to the desulfonated diacetylated derivative. Similarly, the CI spectrum consists primarily of protonated ions at m/z 229 and 271 with a smaller ion at 313. The ions observed at m/z 64 and 48 (EI) and m/z 65 (CI) indicate $SO_2$ is formed under MS conditions.

## 5. CONCLUSIONS

One- and two-dimensional NMR experiments, together with mass spectrometry and derivatization and degradation of the actual material, have resulted in an unequivocal assignment of the structure of Compound 34. The structure and the $^1H$ and $^{13}C$ NMR assignments are shown below.




| | $^1H$ Assignments | $^{13}C$ Assignments |
|---|---|---|
| 1 | - | 120.8 |
| 2 | - | 140.0 |
| 3 | 7.34 (J=8.7) | 115.0 |
| 4 | 7.17 (J=8.7,2.6) | 124.4 |
| 5 | - | 134.5 |
| 6 | 7.70 (J=2.6) | 122.7 |
| $NH_3^+$ | 9.69 | - |
| NH | 8.66 | - |
| $CH_3$ | 1.33 (J=7.0) | 14.7 |
| $CH_2$ | 4.01 (J=7.0) | 64.0 |
| 1' | - | 131.2 |
| 2' | - | 148.6 |
| 3' | 7.00 | 113.0 |
| 4' | 6.88 | 121.3 |
| 5' | 6.89 | 120.6 |
| 6' | 7.28 | 116.3 |

Compound 34 is an N-phenyl-para-phenylenediamine with ethoxy and sulfonic acid substituents. The NMR showed that the sulfonic acid group is on the ring with the $-NH_2$ moiety and that it is meta to this group. The ethoxy is on the other ring, ortho to the NH(phenyl) moiety. Therefore, the correct chemical name for compound 34 is either 5-amino-2(2'-ethoxyphenyl)aminobenzenesulfonic acid or 4-amino-2'-ethoxy-2-sulfo diphenylamine.

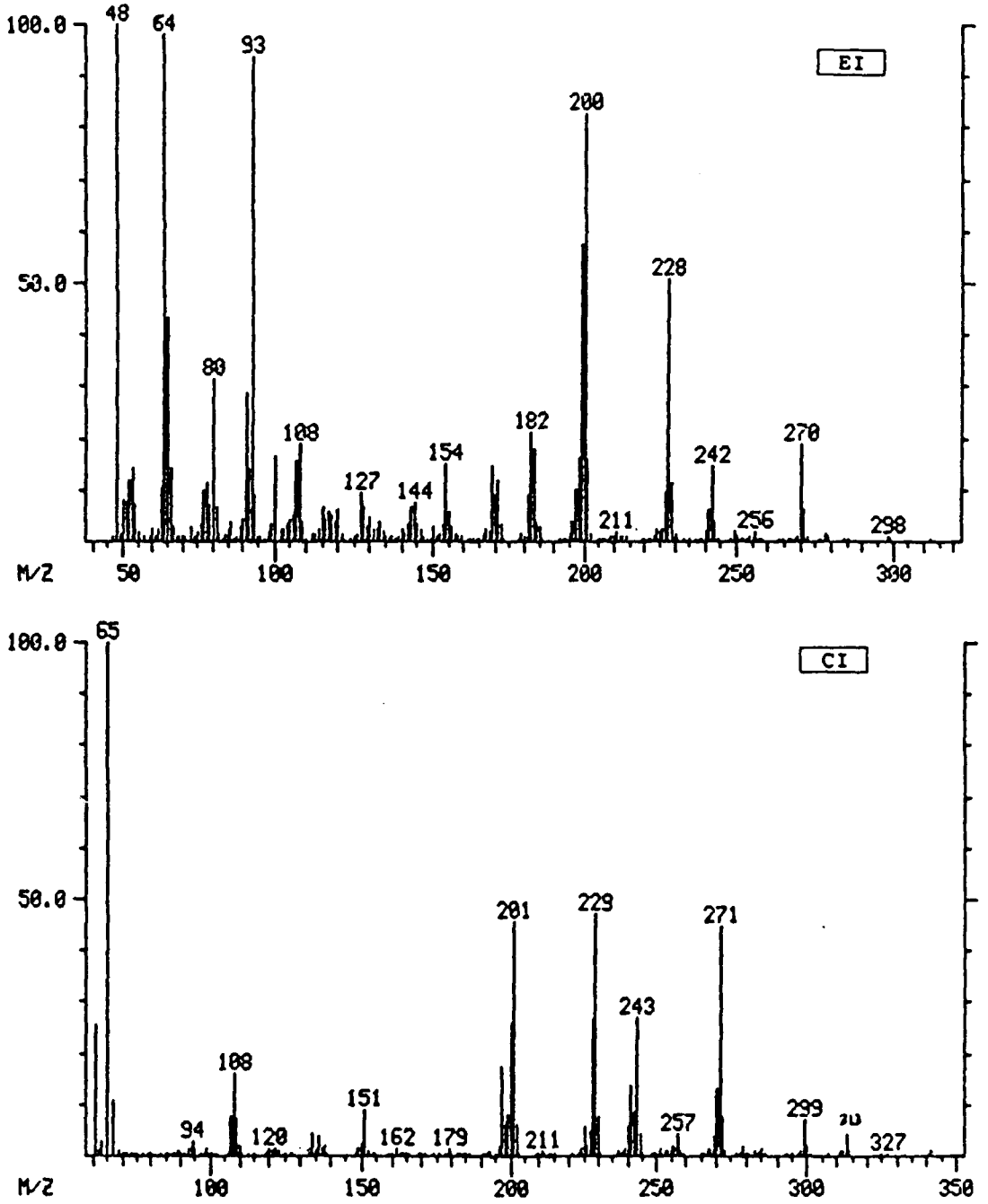



Figure 23. Diacetylated Compound 34 (VI): EI and CI Mass Spectra

The CRDEC sample of Compound 34 was found to be >95% pure. TLC showed two small impurities; one was highly colored and accounted for the bluish-purple color of the sample.

Blank

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